Lights of Tuscany 2022

Towards combining lasers and gene editing via plasmonics

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I·Gene

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862714.

OUTLOOK

Towards combining lasers and gene editing via plasmonics

Three keywords:

1. Lasers:
   which are the most relevant features for the experiment?

2. Gene editing:
   what is that?

3. Plasmonics:
   how can it help?

OUTLOOK

Towards combining lasers and gene editing via plasmonics

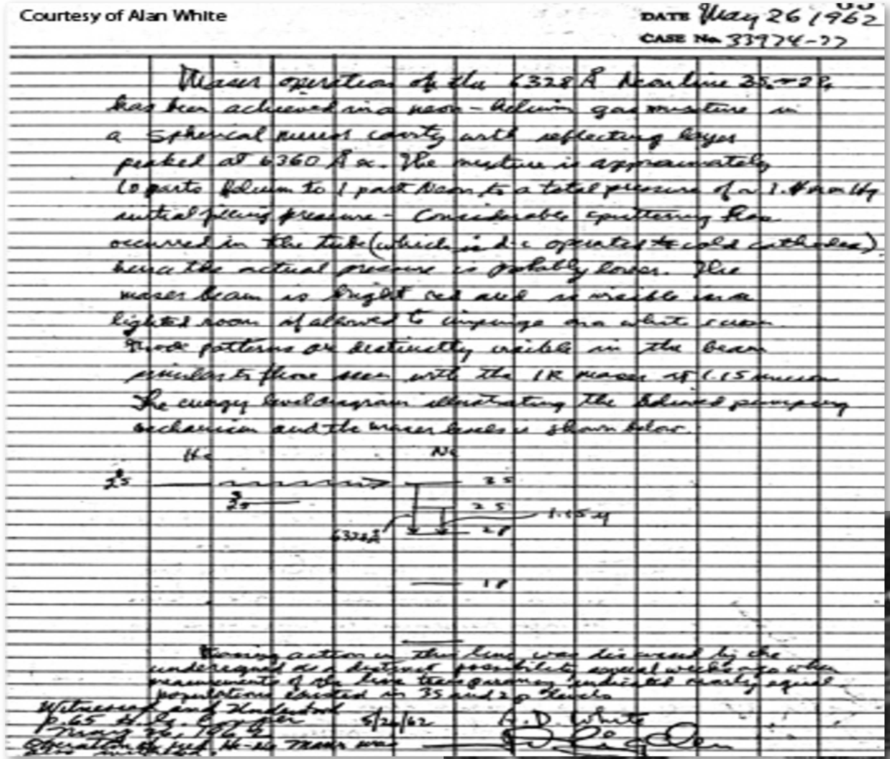
Three keywords:

1. Lasers:
   which are the most relevant features for the experiment?
2. Gene editing:
   what is that?
3. Plasmonics:
   how can it help?

preliminary experimental results, so far…

LASER

A.White's notebook on the first realization of a HeNe laser



Courtesy of Alan White

DATE May 26 1962
CASE No. 33974-77

Maser operation of the 6328 Å Neon line $3S_2 \rightarrow 2p_4$ has been achieved in a neon-helium gas mixture in a spherical mirror cavity with reflecting layers peaked at 6360 Å ... The mixture is approximately 10 parts helium to 1 part Neon to a total pressure of ~ 1.4 mm Hg initial filling pressure. Considerable sputtering has occurred in the tube (which is d.c. operated with cold cathodes) hence the actual pressure is probably lower. The maser beam is bright red and is visible in a lighted room if allowed to impinge on a white screen. Mode patterns are distinctly visible in the beam similar to those seen with the IR maser at 1.15 micron. The energy level diagram illustrating the believed pumping mechanism and the maser levels is shown below.

He   Ne
2^1S 3S
2^3S 2S
1.15 μ
6328 Å   2P
1P

Maser action in this line was discussed by the undersigned as a distinct possibility several weeks ago when measurements of the line transparency indicated nearly equal populations existed in 3S and 2p levels.

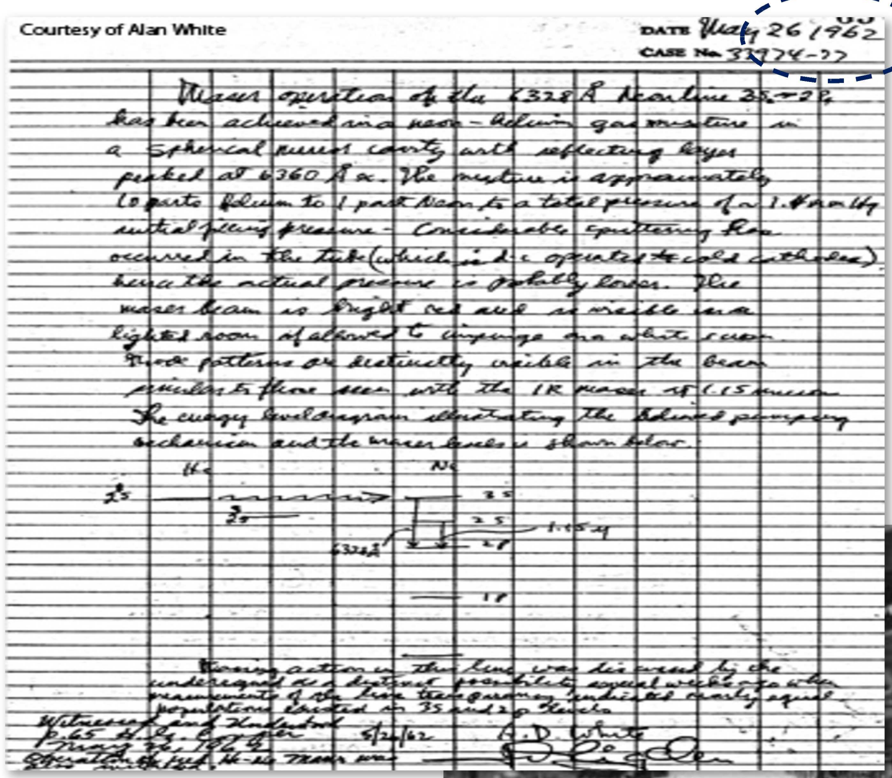
Witnessed and Understood
[illegible]
May 26, 1962
Operation of the He-Ne maser was [illegible]

5/26/62   A.D. White

J. Hecht, Opt. Eng. 49 01002 (2010)

LASER

A.White's notebook on the first realization of a HeNe laser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A solution seeking a problem


THE NEW YORK TIMES, WEDNESDAY, MAY 6, 1964

Developer of the Laser Calls It 'A Solution Seeking a Problem'

$10,614,000,000 Refunding Offer to Holders of U.S. Treasury Securities

The First Boston Corporation

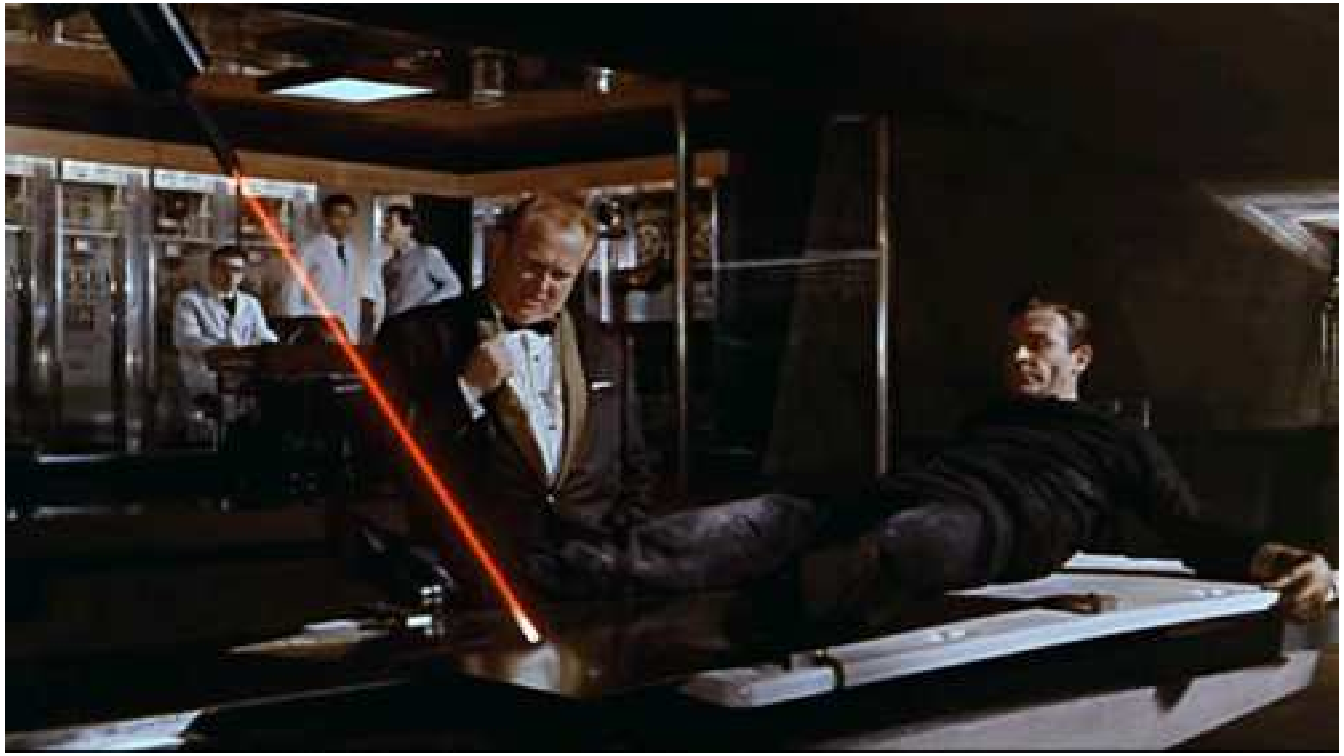
EACH YEAR YOU EARN 4.97%

WHEN OUR 4.85% IS COMPOUNDED

INSURED

LASER

A problem found and solved



Goldfinger - 1964

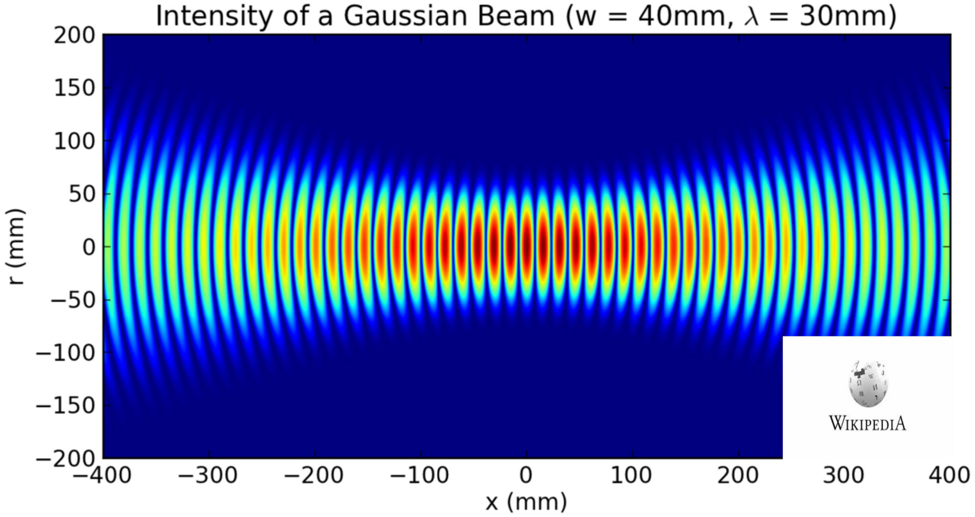
LASER

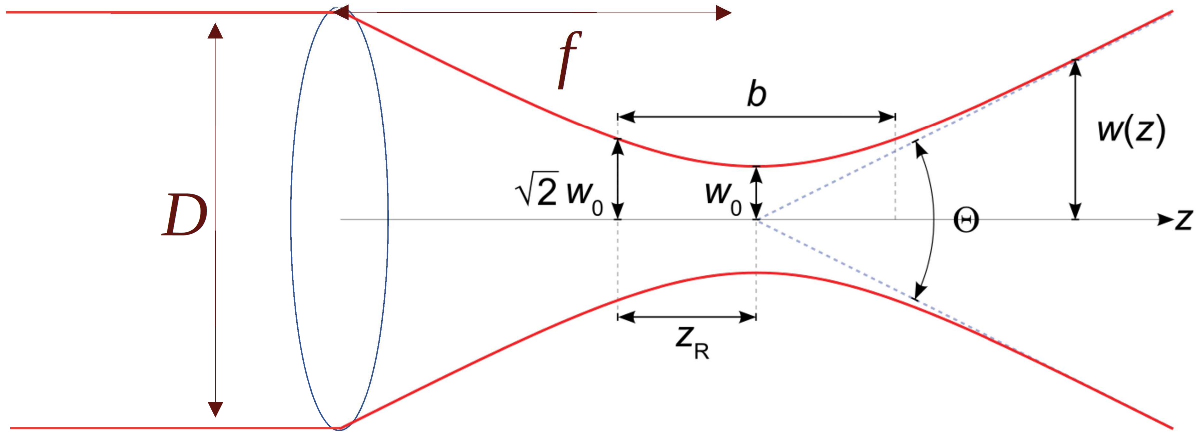
Laser as an extremely intense source of energy

Goldfinger - 1964

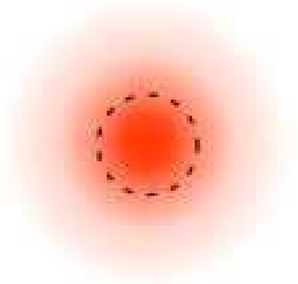
LASER

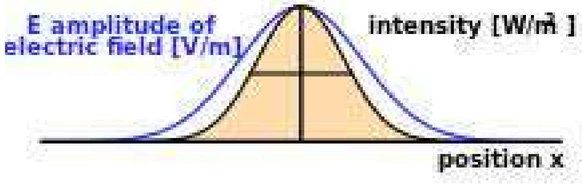
$$I = \frac{<P>}{A}$$





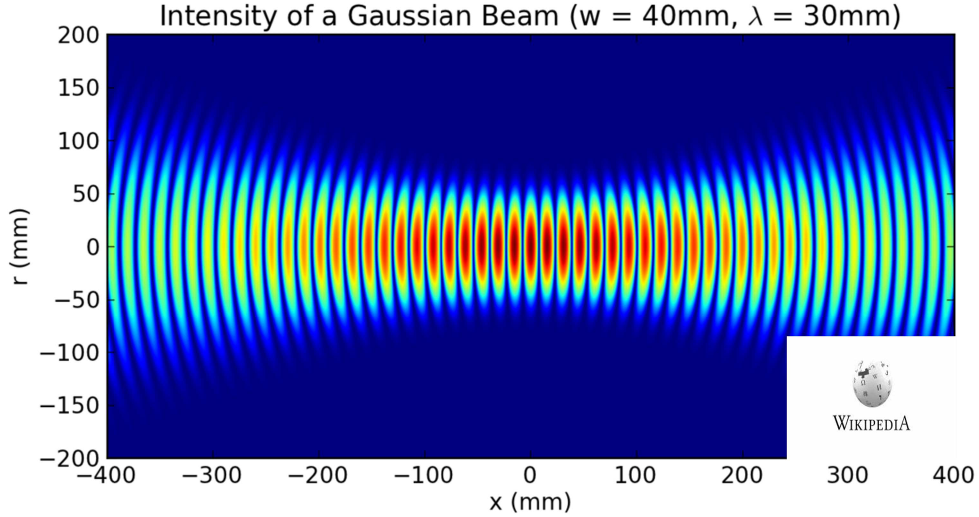
$$2w_0 \simeq M^2 \frac{2\lambda}{\pi\theta} \simeq M^2 \frac{4\lambda f}{\pi D}$$

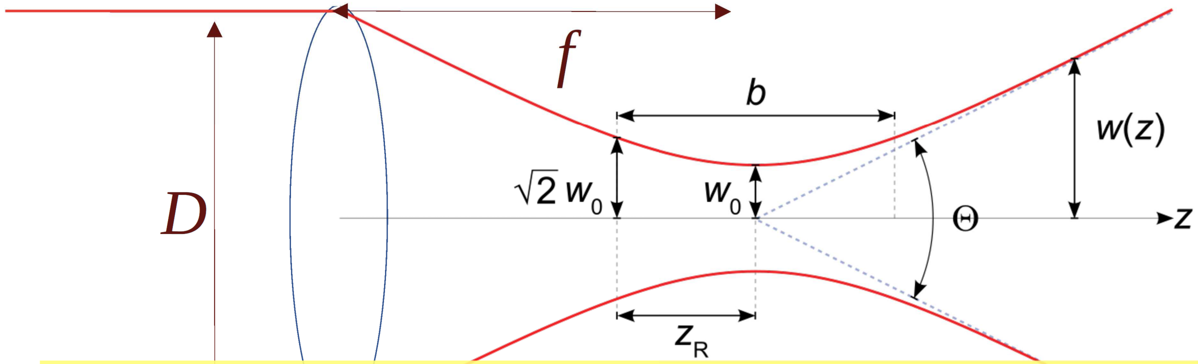


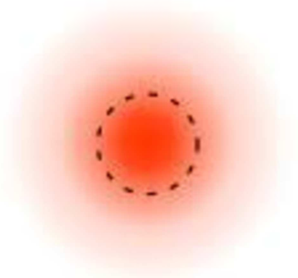


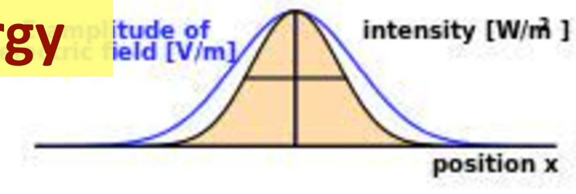
LASER

$$I = \frac{<P>}{A}$$









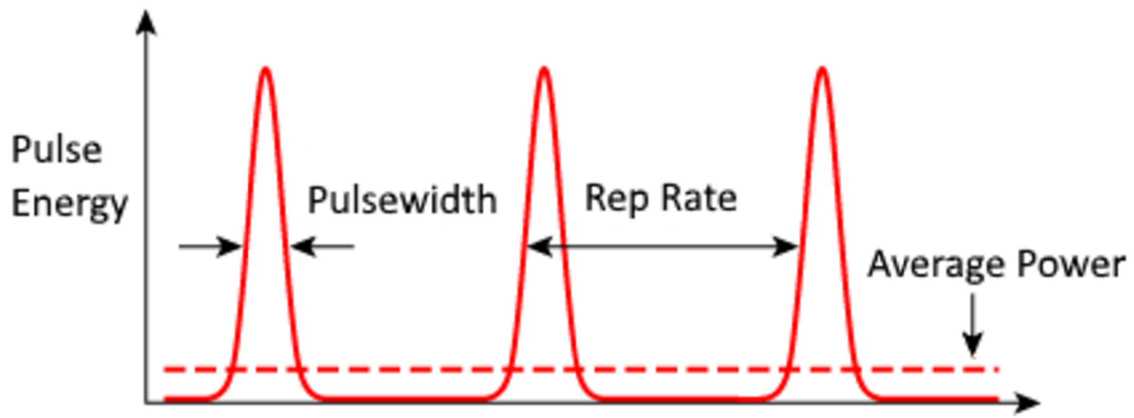
Laser as an extremely localized source of energy

$$2w_0 \simeq M^2 \frac{2\lambda}{\pi\theta} \simeq M^2 \frac{4\lambda f}{\pi D}$$

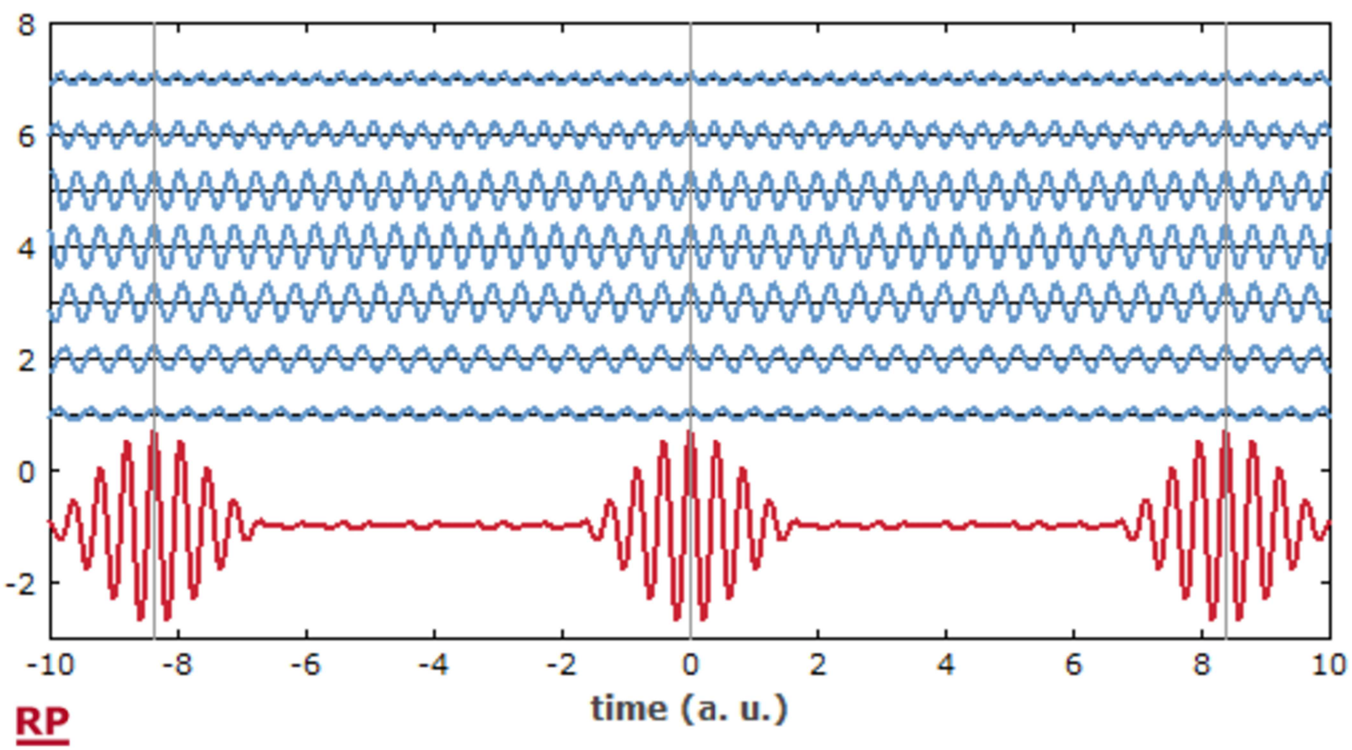
LASER

Pulsed lasers

$$I = \frac{<E>/\Delta t}{A}$$



Mode locking



LASER

Pulsed lasers

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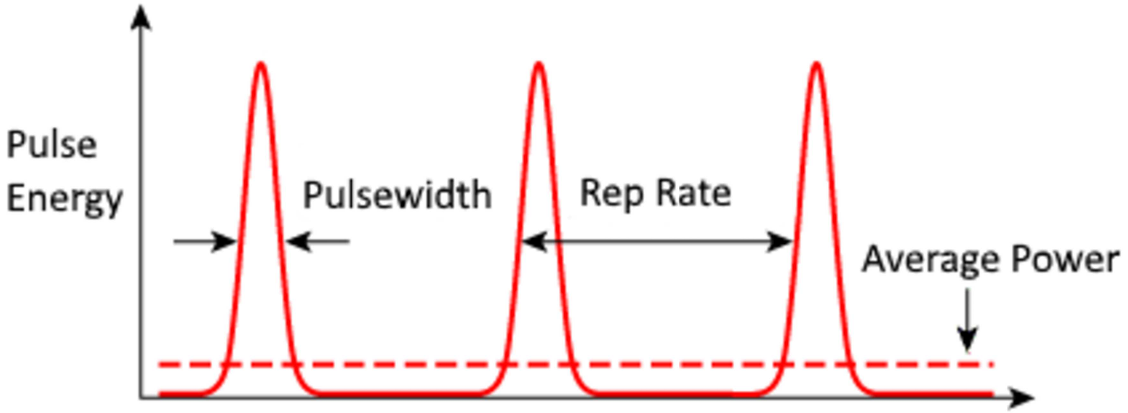
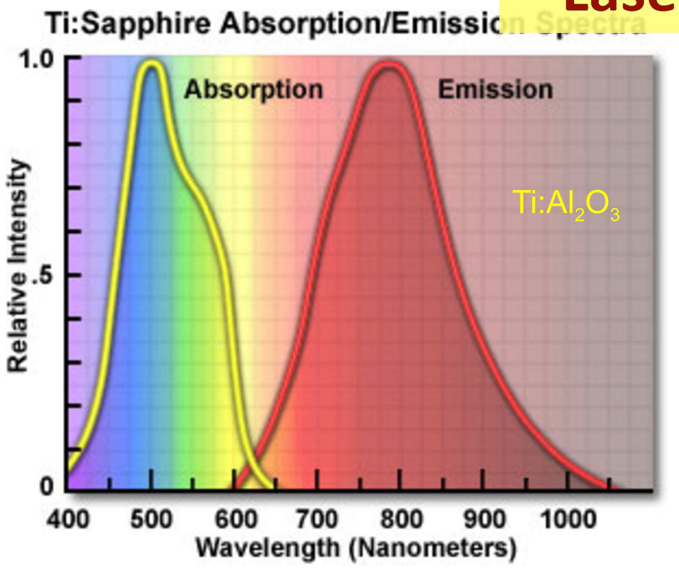
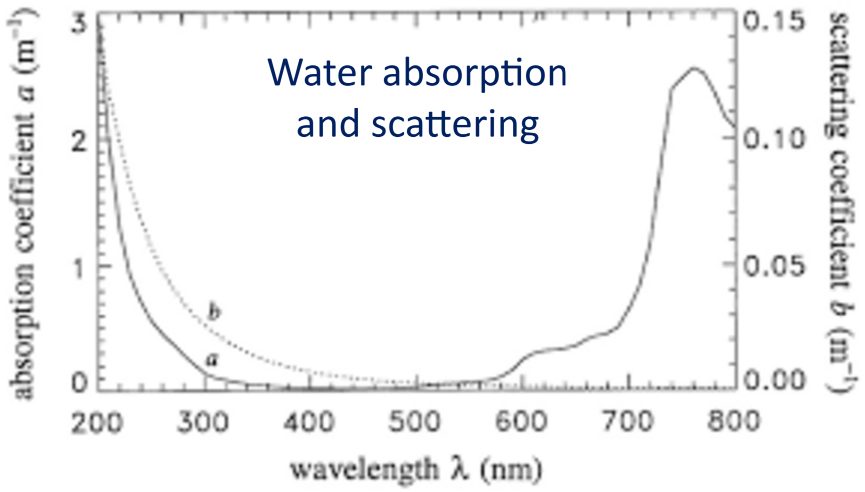
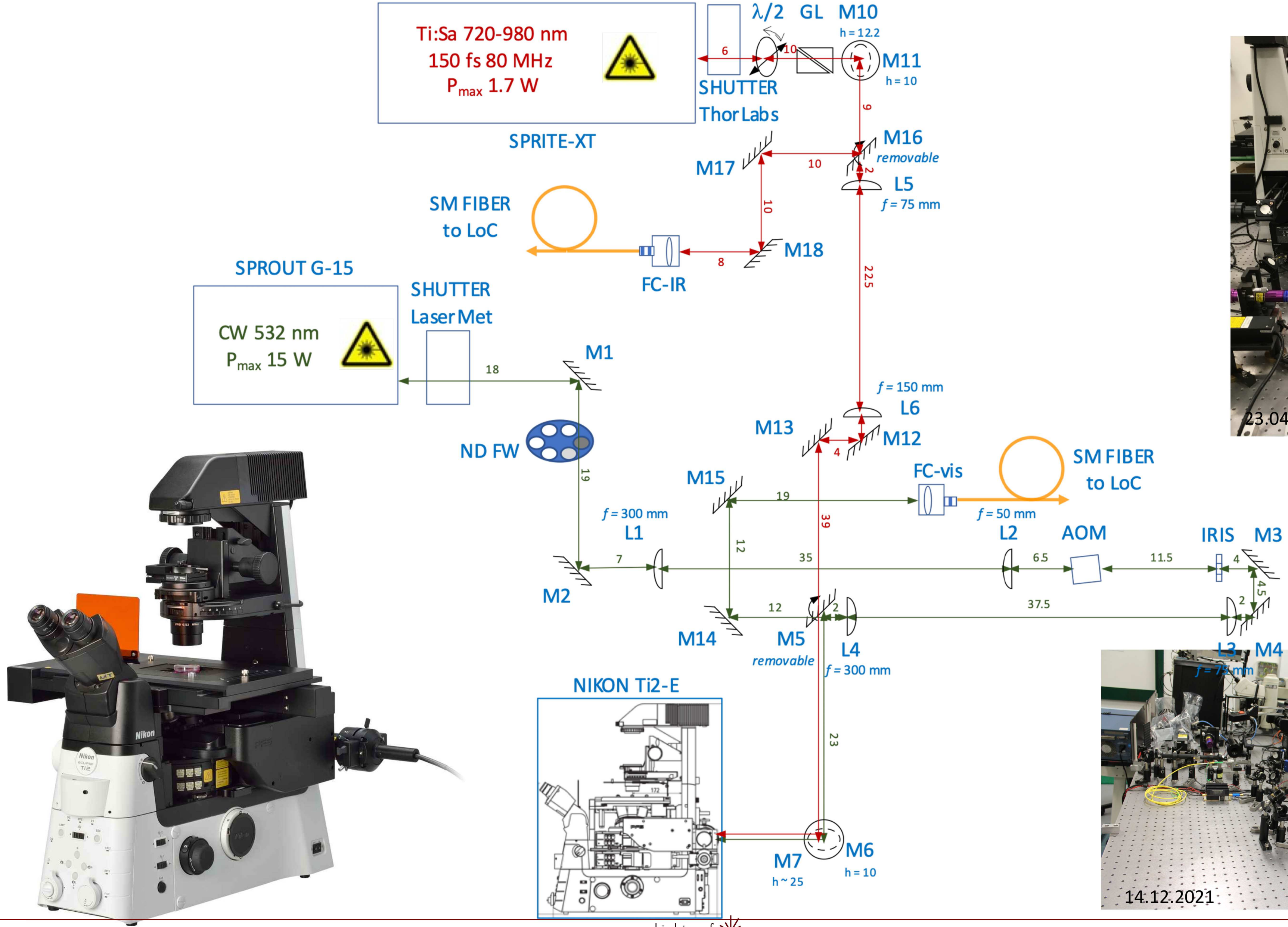


Laser as a temporally localized source of energy

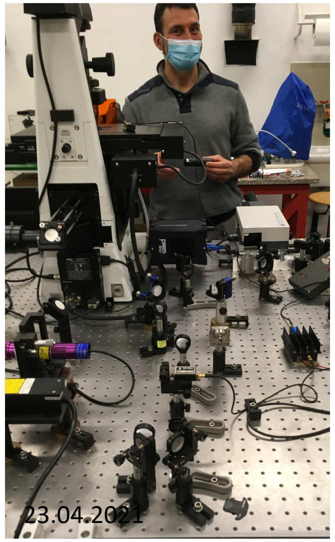




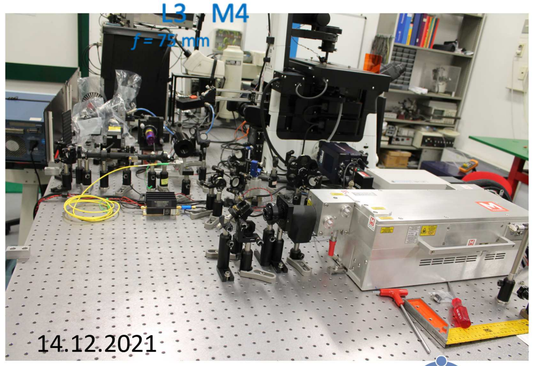
SETUP



Ti:Sa 720-980 nm
150 fs 80 MHz
P_{max} 1.7 W
SPRITE-XT
SHUTTER
ThorLabs
λ/2
GL
M10
h = 12.2
M11
h = 10
M16
removable
M17
L5
f = 75 mm
SM FIBER
to LoC
FC-IR
M18
SPROUT G-15
CW 532 nm
P_{max} 15 W
SHUTTER
LaserMet
M1
ND FW
f = 150 mm
L6
M13
M12
M15
FC-vis
SM FIBER
to LoC
f = 300 mm
L1
f = 50 mm
L2
AOM
IRIS
M3
M2
M14
M5
removable
L4
f = 300 mm
L3
f = 75 mm
M4
NIKON Ti2-E
M7
h ~ 25
M6
h = 10
6
10
9
10
2
10
8
22.5
18
19
4
19
39
12
7
35
6.5
11.5
4
4.5
12
2
37.5
2
23

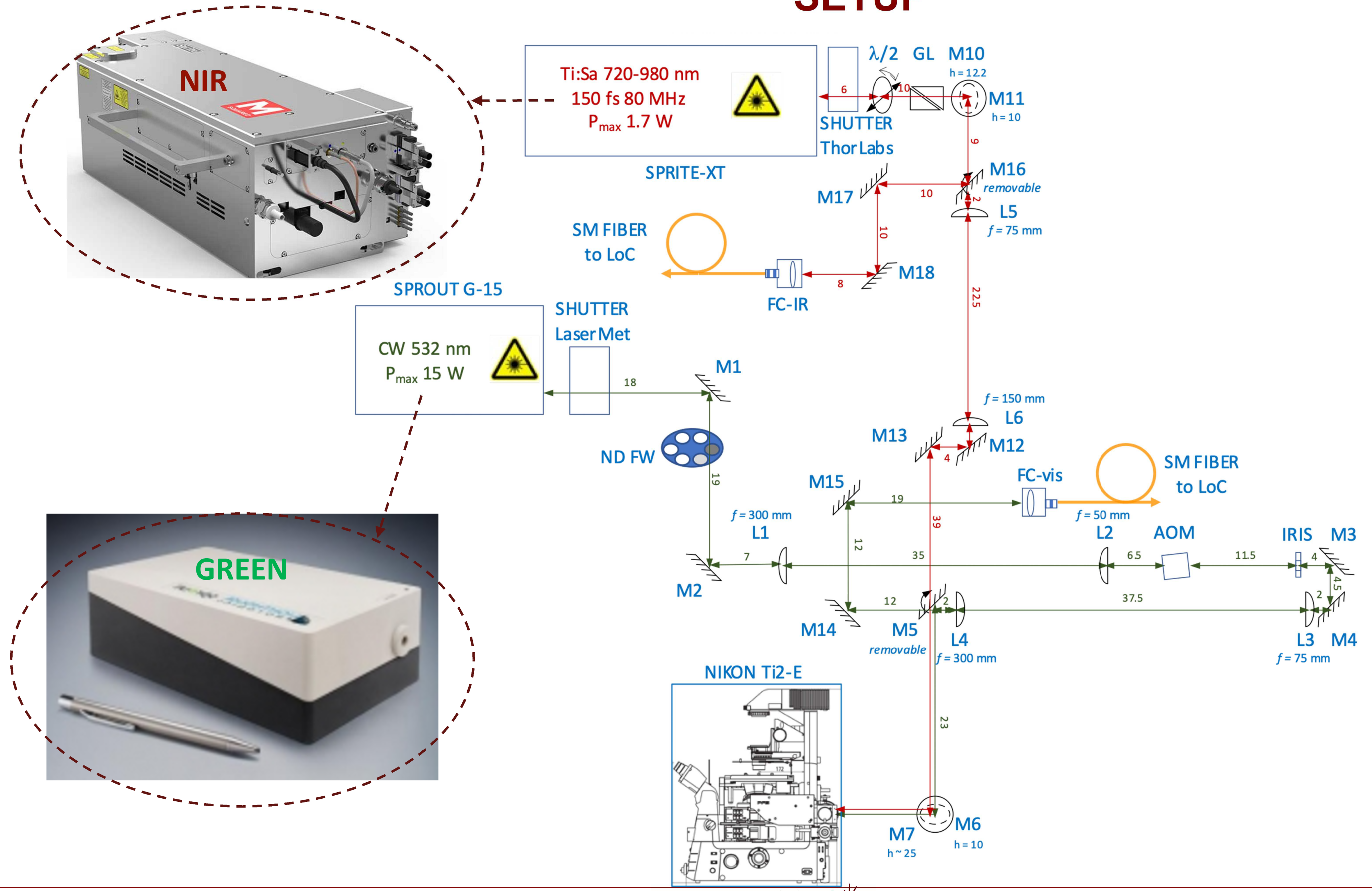


23.04.2021

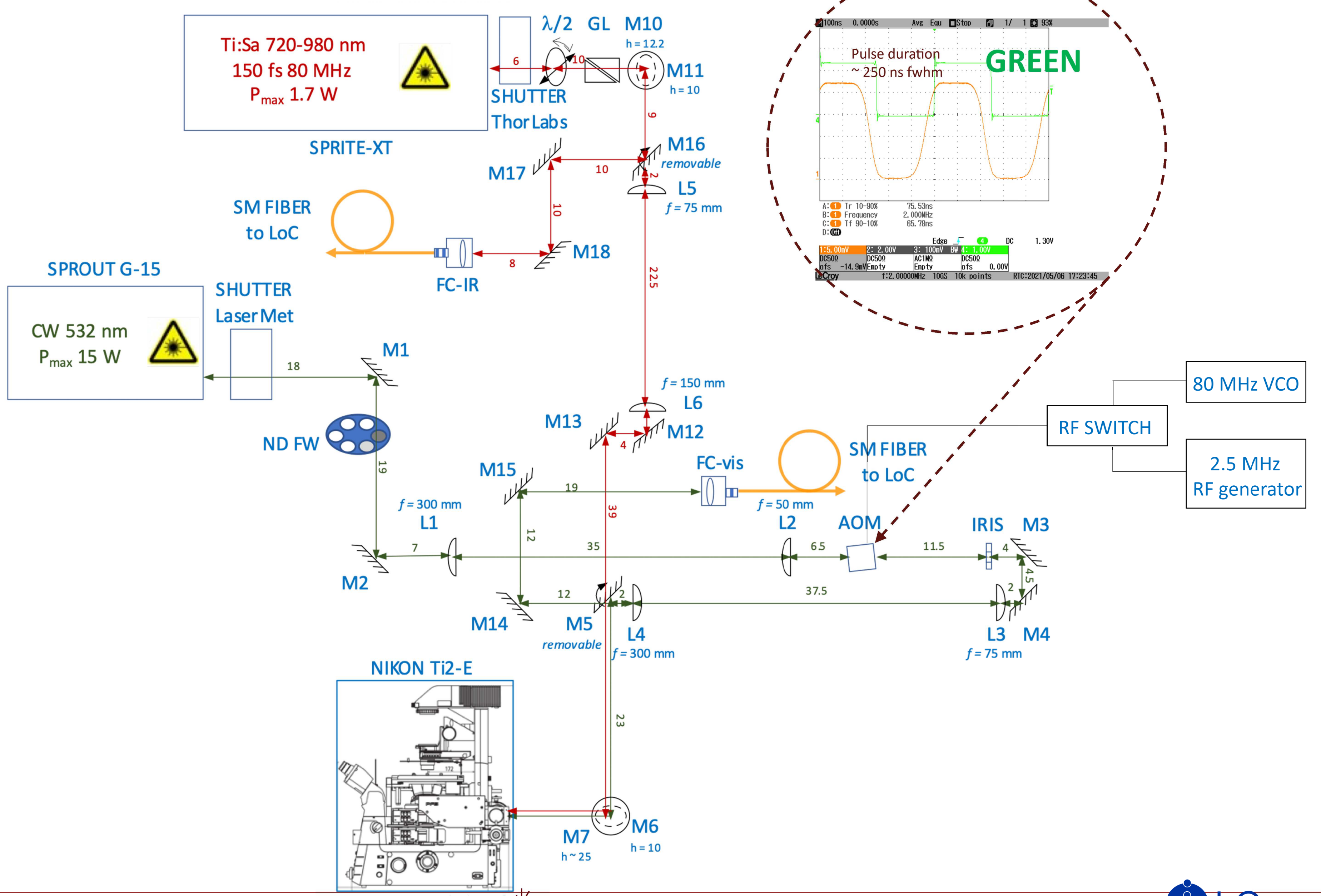


14.12.2021

SETUP

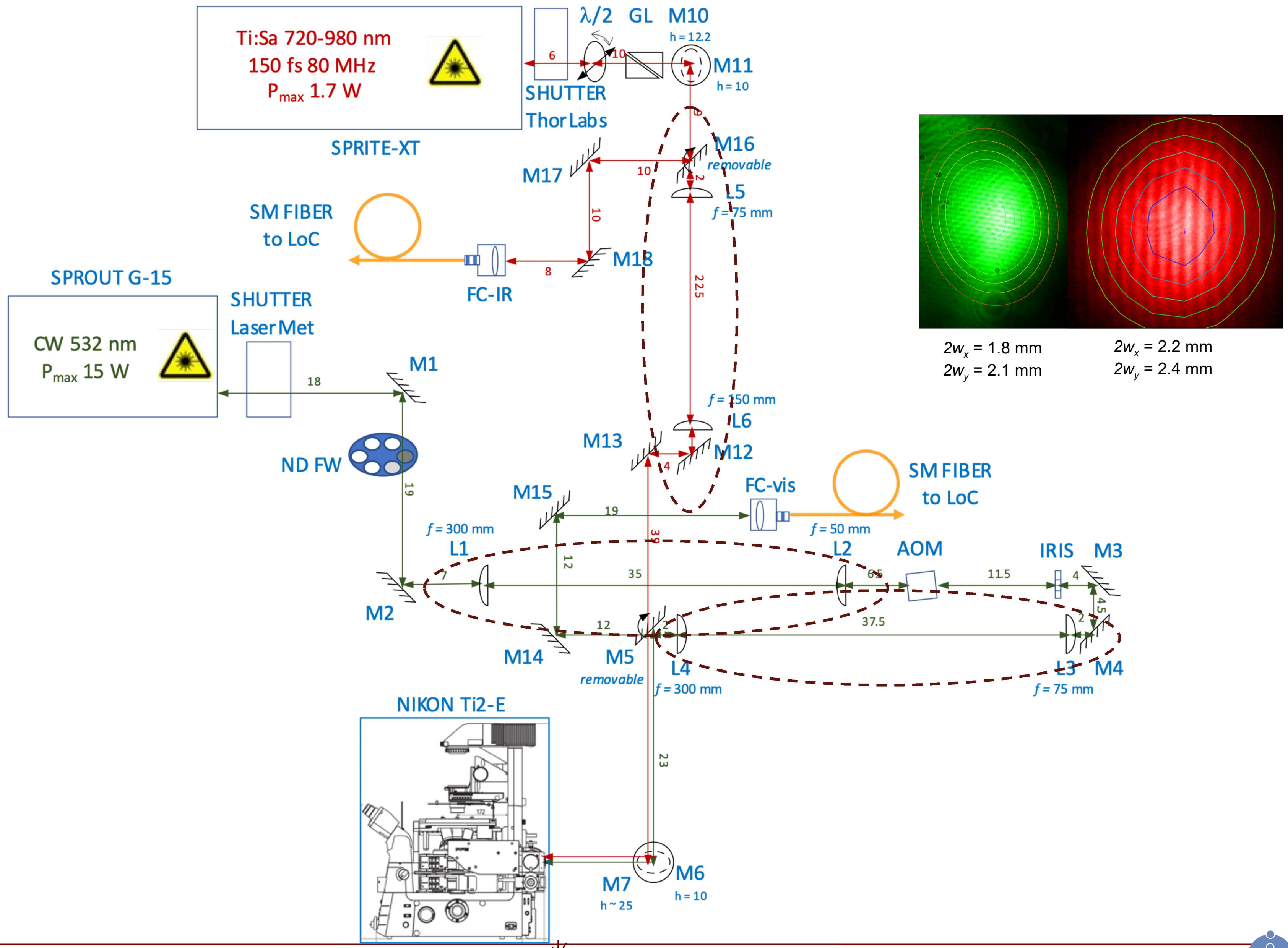

NIR
Ti:Sa 720-980 nm
150 fs 80 MHz
P max 1.7 W
SPRITE-XT
6
SHUTTER
Thor Labs
λ/2
10
GL
M10
h = 12.2
M11
h = 10
9
M16
removable
2
L5
f = 75 mm
M17
10
10
M18
8
FC-IR
SM FIBER
to LoC
22.5
SPROUT G-15
CW 532 nm
P max 15 W
SHUTTER
Laser Met
18
M1
ND FW
19
GREEN
f = 150 mm
L6
M13
4
M12
FC-vis
SM FIBER
to LoC
M15
19
39
f = 300 mm
L1
12
7
35
M2
f = 50 mm
L2
6.5
AOM
11.5
IRIS
4
M3
4.5
2
12
M14
M5
removable
2
L4
f = 300 mm
37.5
L3
f = 75 mm
M4
NIKON Ti2-E
23
M7
h ~ 25
M6
h = 10

SETUP



Ti:Sa 720-980 nm
150 fs 80 MHz
Pmax 1.7 W
SPRITE-XT
SHUTTER
Thor Labs
λ/2
GL
M10
h = 12.2
M11
h = 10
M16
removable
L5
f = 75 mm
M17
M18
FC-IR
SM FIBER
to LoC
SPROUT G-15
CW 532 nm
Pmax 15 W
SHUTTER
Laser Met
M1
ND FW
M2
L1
f = 300 mm
M15
M14
M13
M12
L6
f = 150 mm
FC-vis
SM FIBER
to LoC
L2
f = 50 mm
AOM
IRIS
M3
M4
L3
f = 75 mm
M5
removable
L4
f = 300 mm
M6
h = 10
M7
h ~ 25
NIKON Ti2-E
RF SWITCH
80 MHz VCO
2.5 MHz
RF generator
Pulse duration
~ 250 ns fwhm
GREEN

SETUP

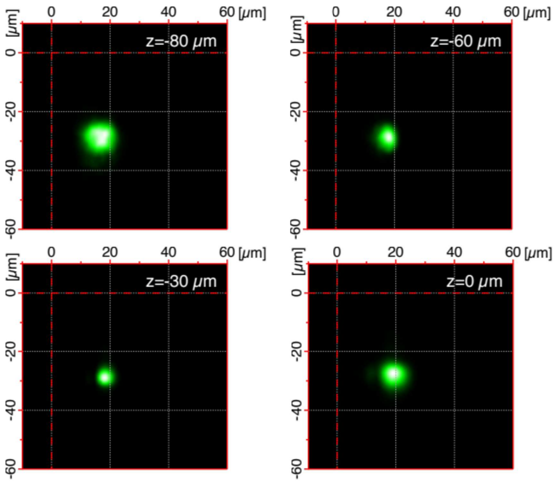


$2w_x$ = 1.8 mm
$2w_y$ = 2.1 mm

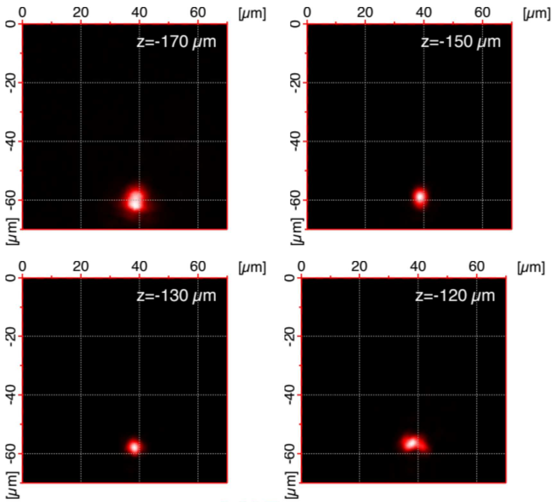
$2w_x$ = 2.2 mm
$2w_y$ = 2.4 mm

SETUP

Images of the focal spot



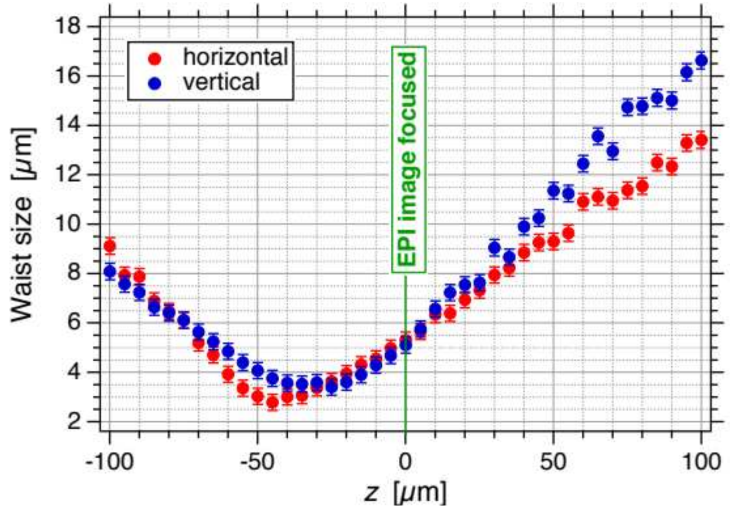
GREEN

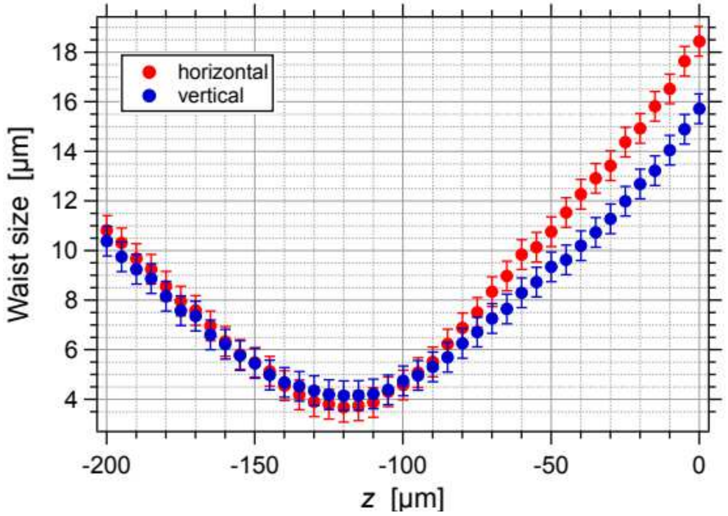


NIR

10x/0.25 objective

Beam envelope





$$I(r,z) = I_0 \left(\frac{w_0}{w(z)} \right)^2 \exp\left(-\frac{2r^2}{w(z)^2} \right)$$

$$w(z) = w_0 \sqrt{1 + \left(\frac{z}{z_R} \right)^2}$$

$$z_R = \frac{\pi w_0^2}{\lambda}$$

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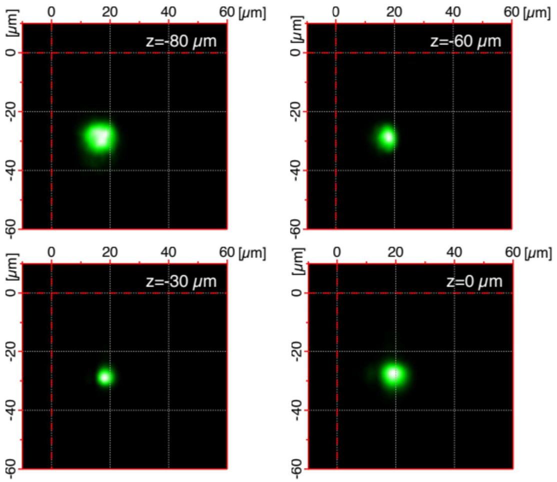
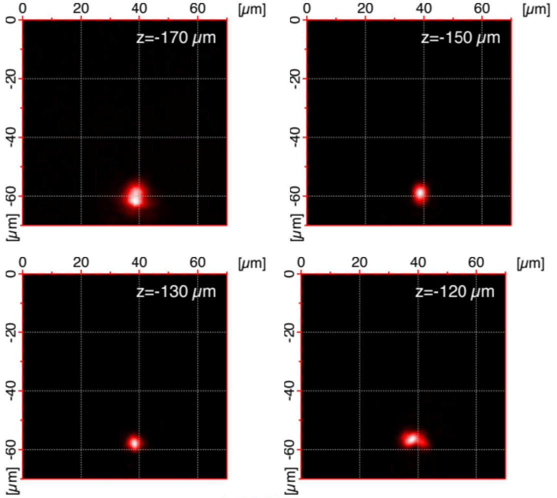
SETUP

Images of the focal spot

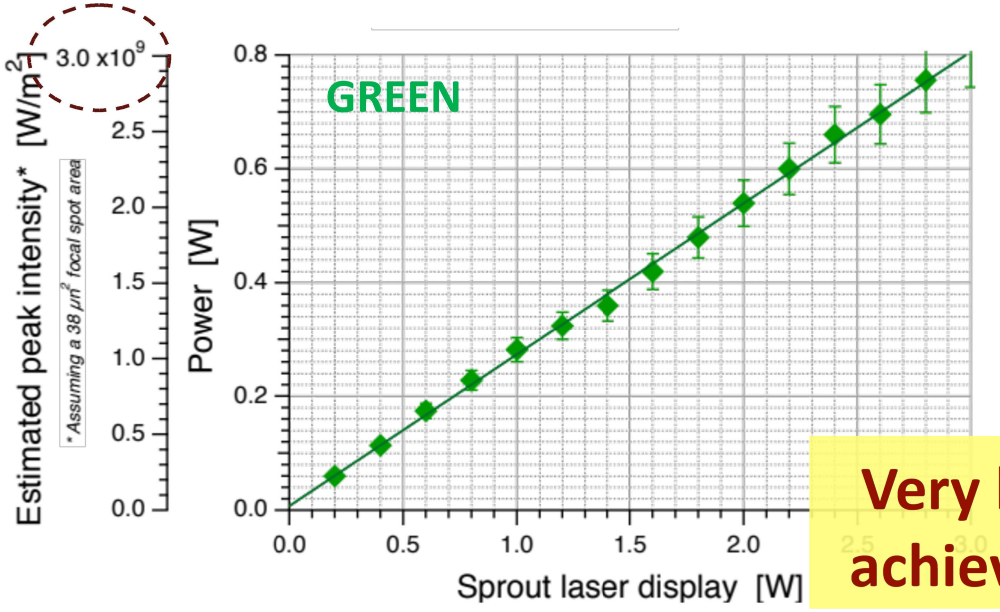


GREEN

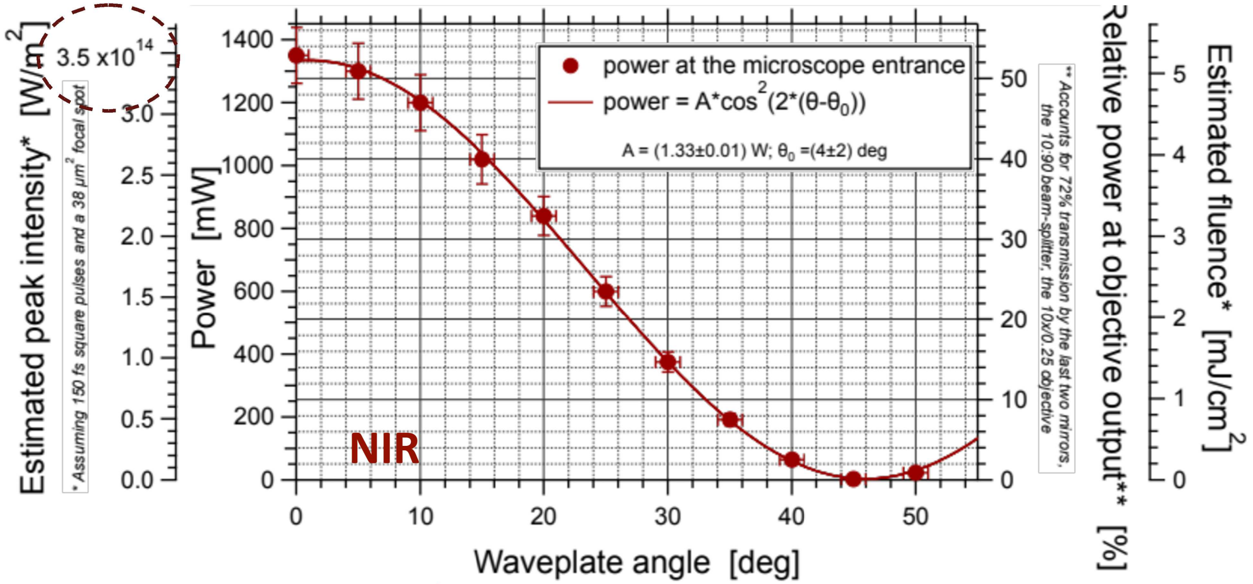


NIR

10x/0.25 objective



Very large laser intensity achieved in the focal spot



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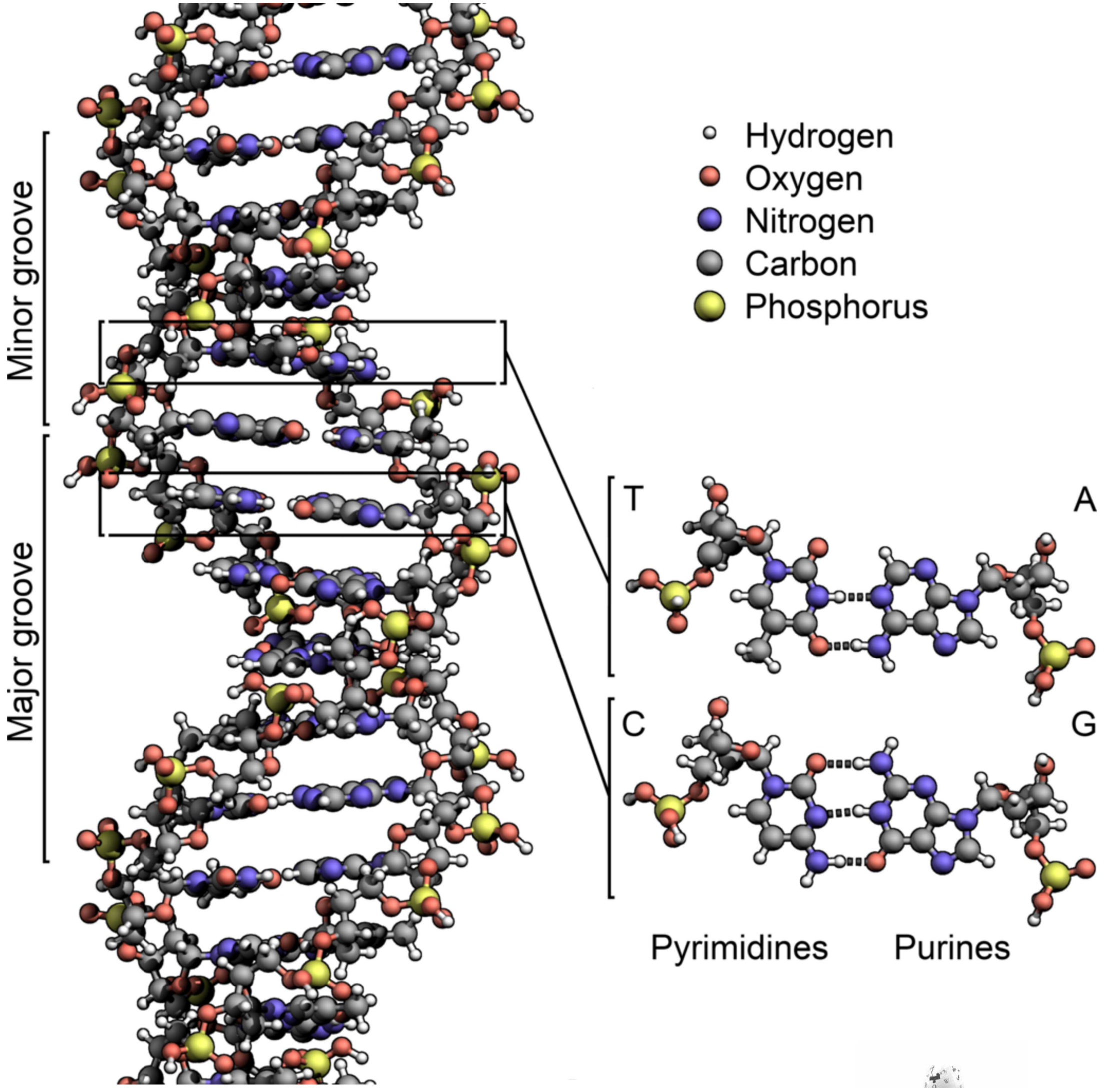
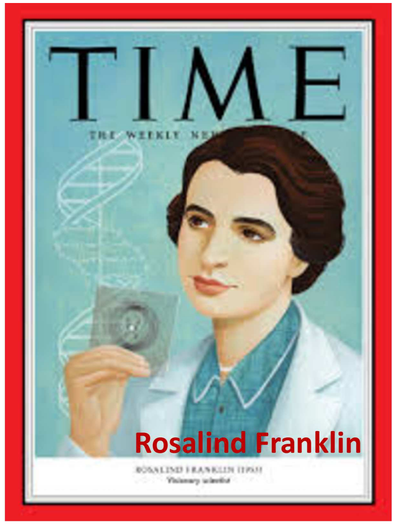
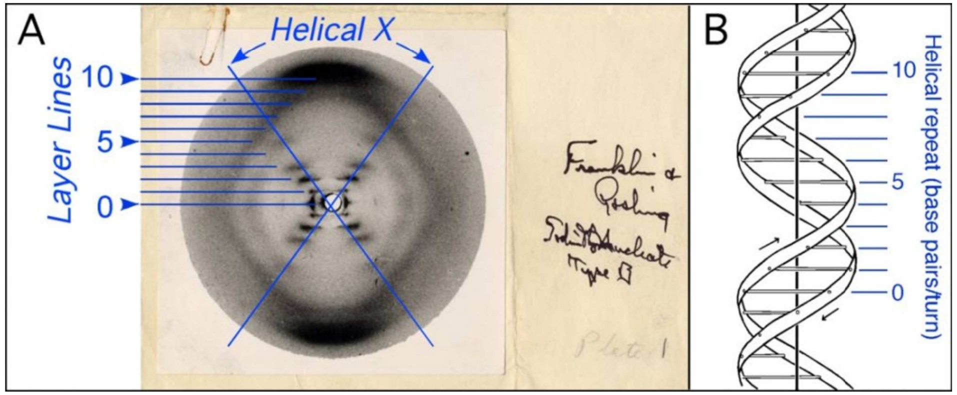
2. GENE EDITING:
WHAT IS THAT?

THE DNA

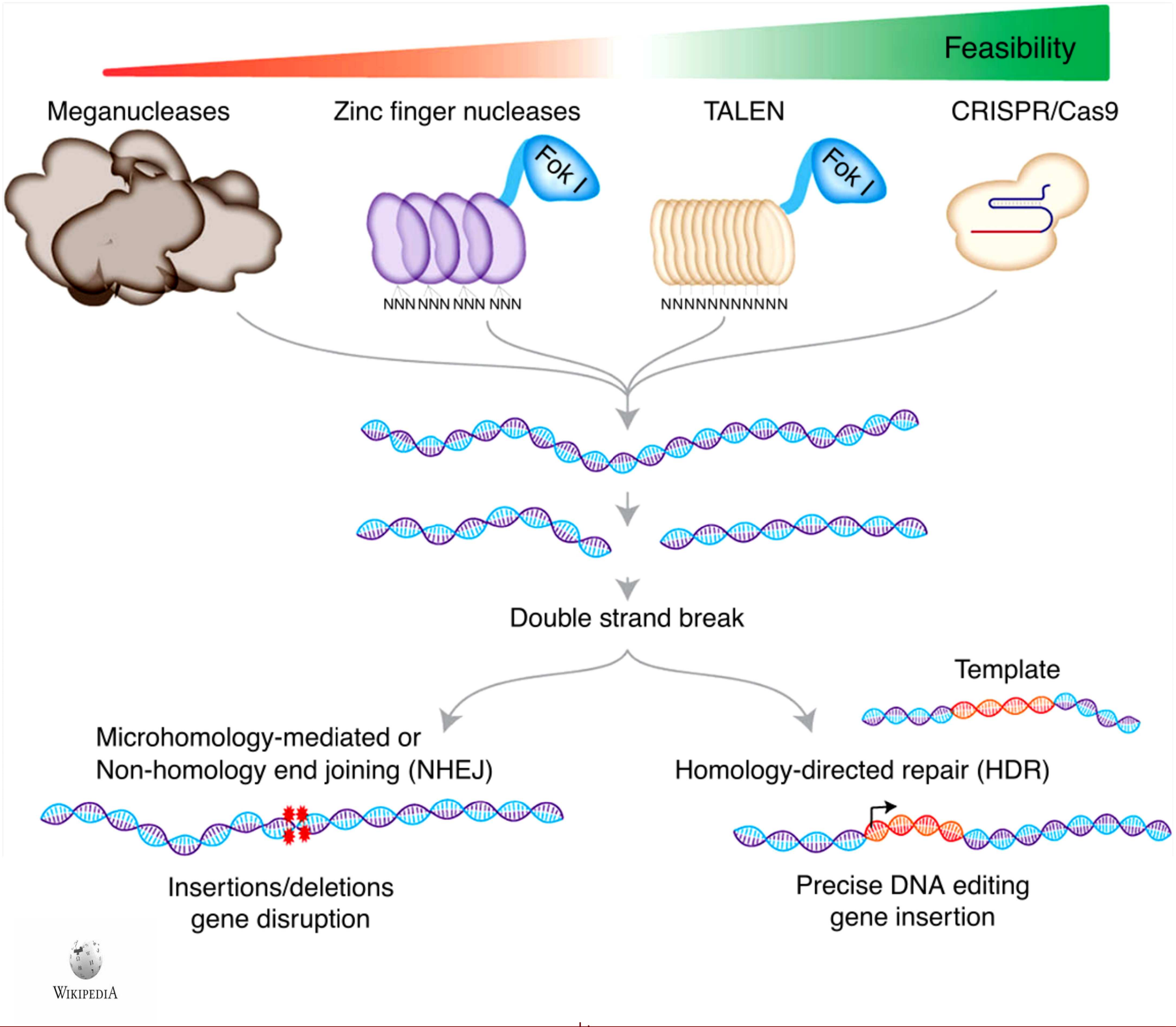




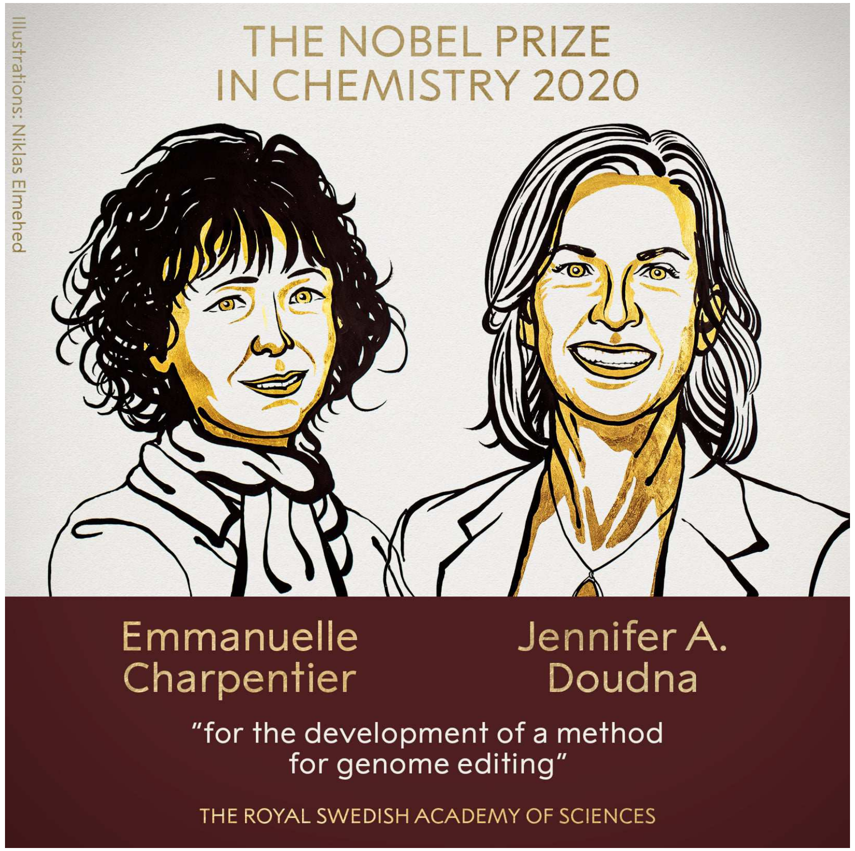


WIKIPEDIA

GENE EDITING



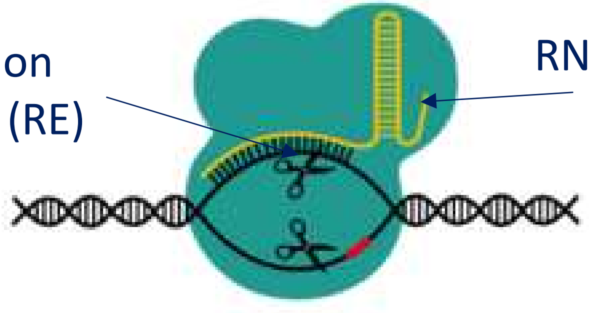
CRISPR-Cas9



Clustered
Regularly
Inter
Spaced
Palindromic
Repeat

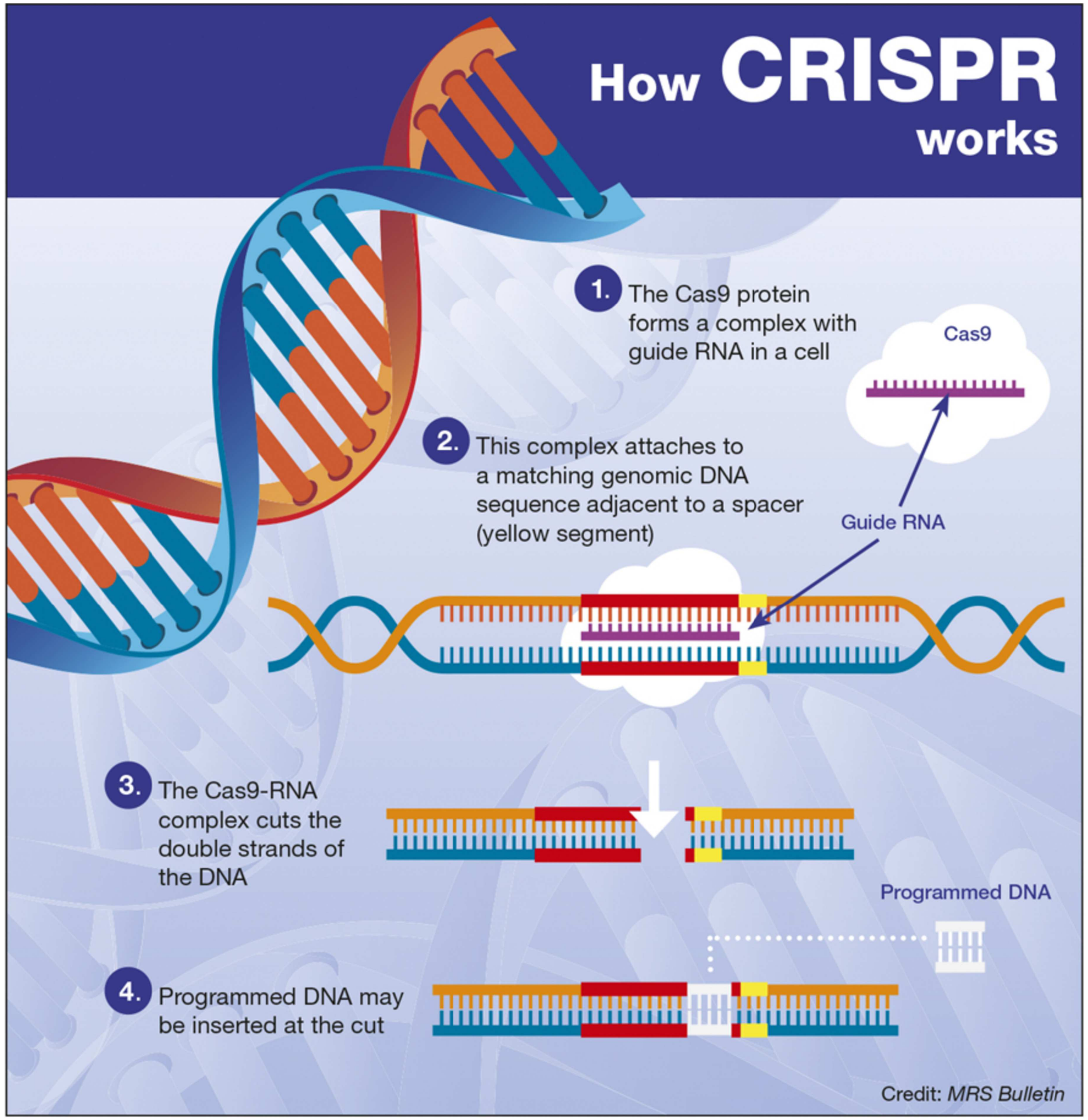
Cas9
(CRISPR associated system) protein

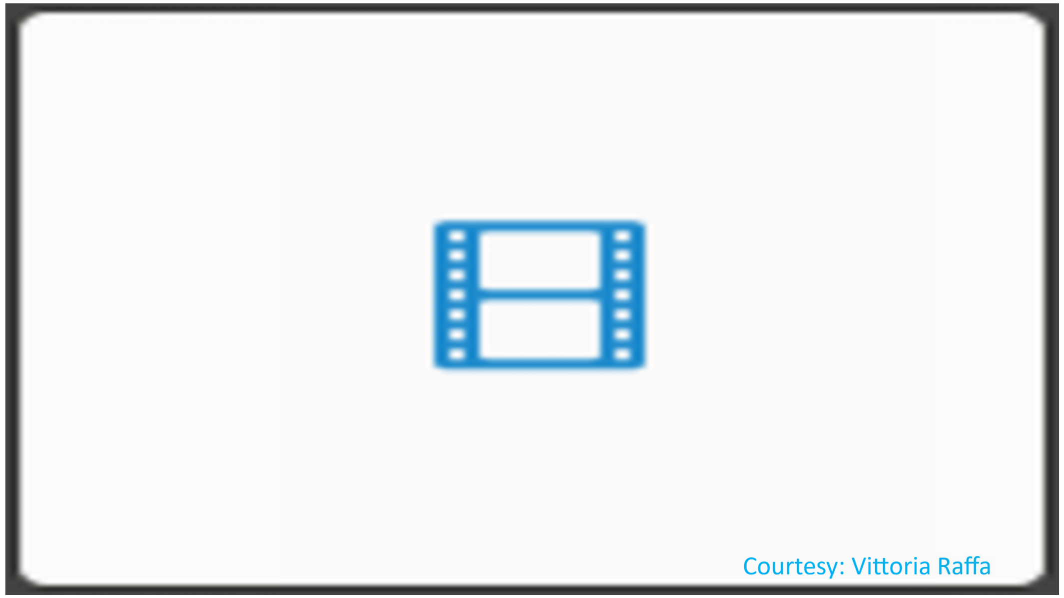
Restriction
Enzyme (RE)



RNA-guide

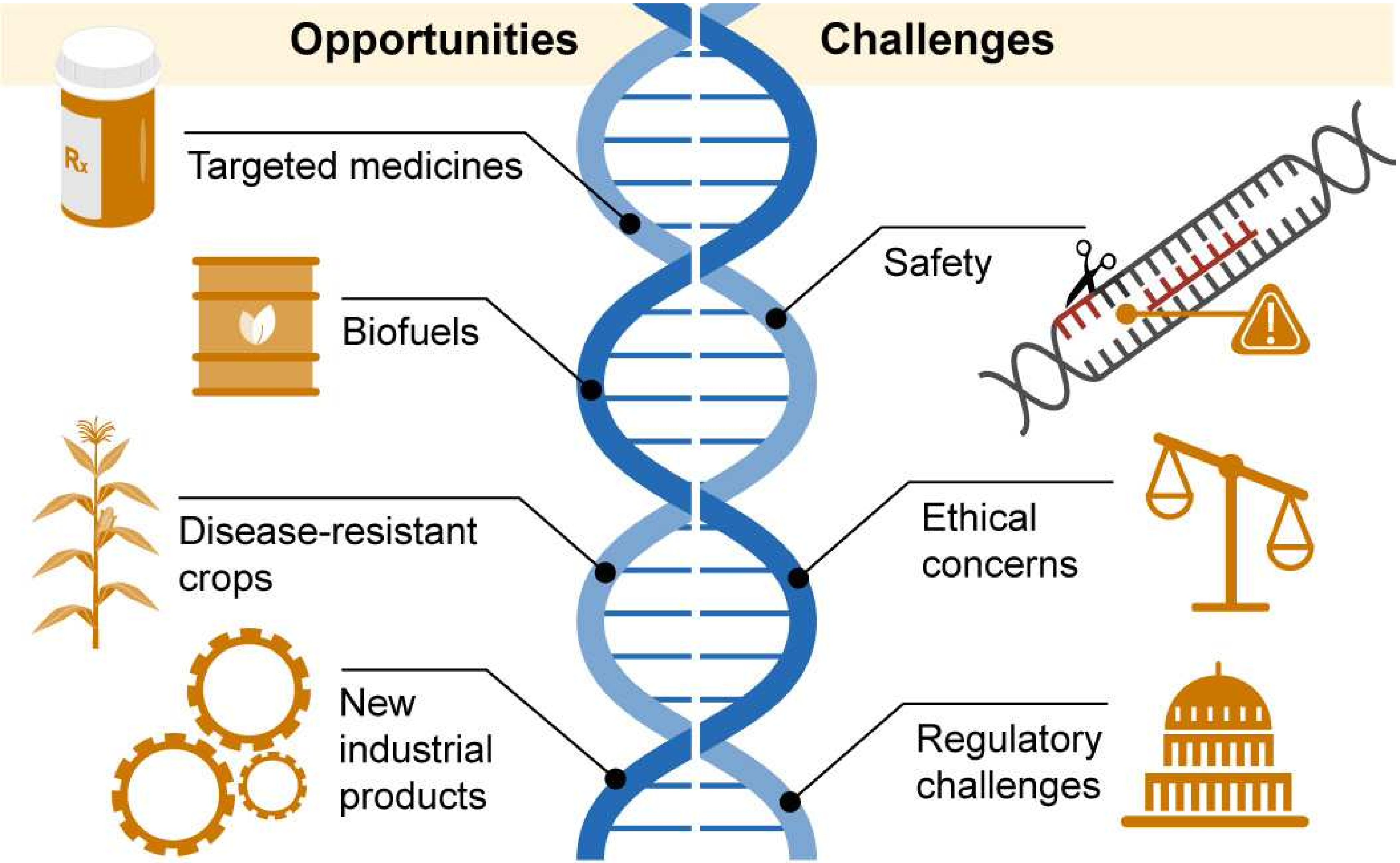
CRISPR-Cas9





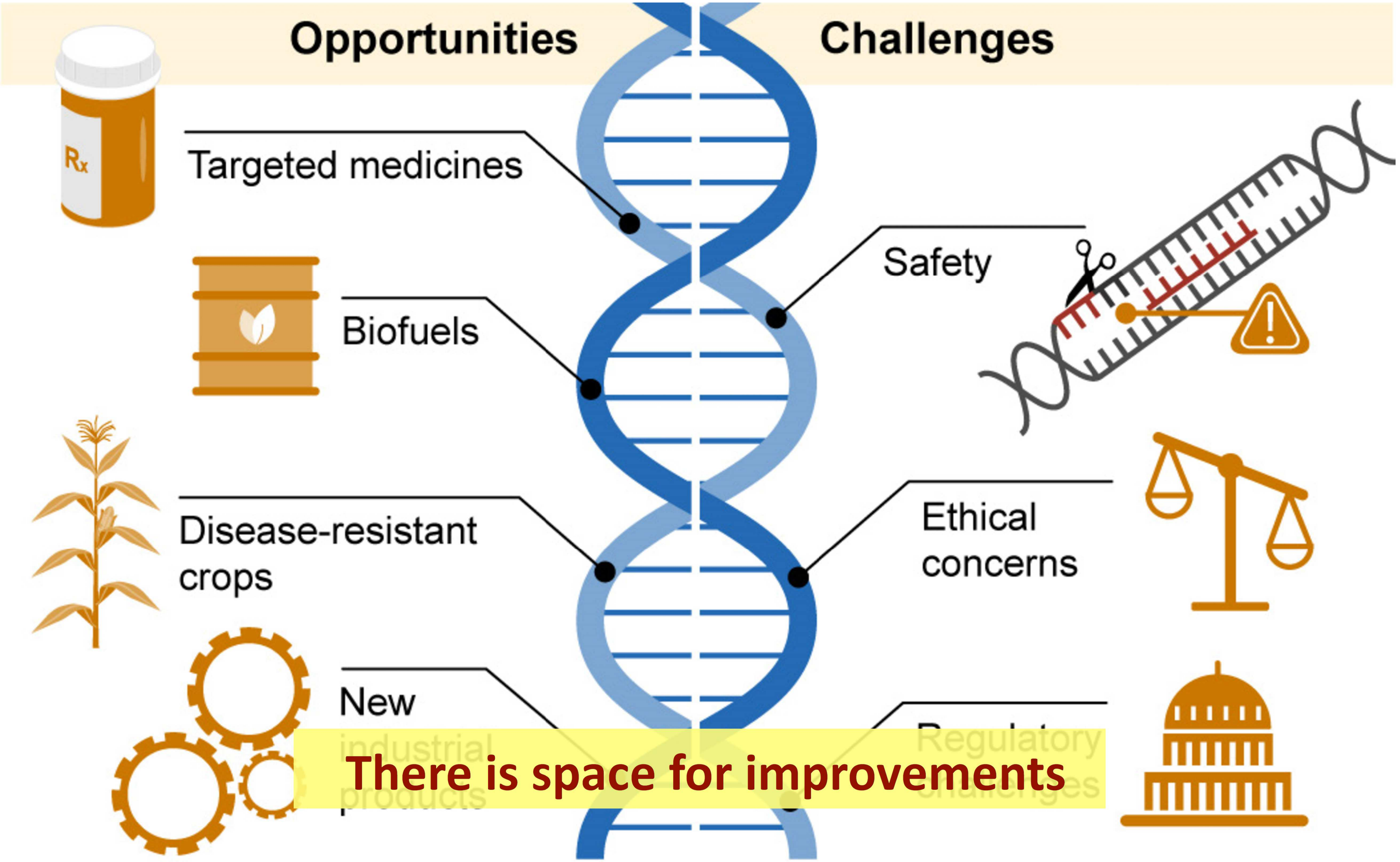
Courtesy: Vittoria Raffa

OPEN ISSUES



Source: GAO. | GAO-20-478SP

OPEN ISSUES



Source: GAO. | GAO-20-478SP

There is space for improvements

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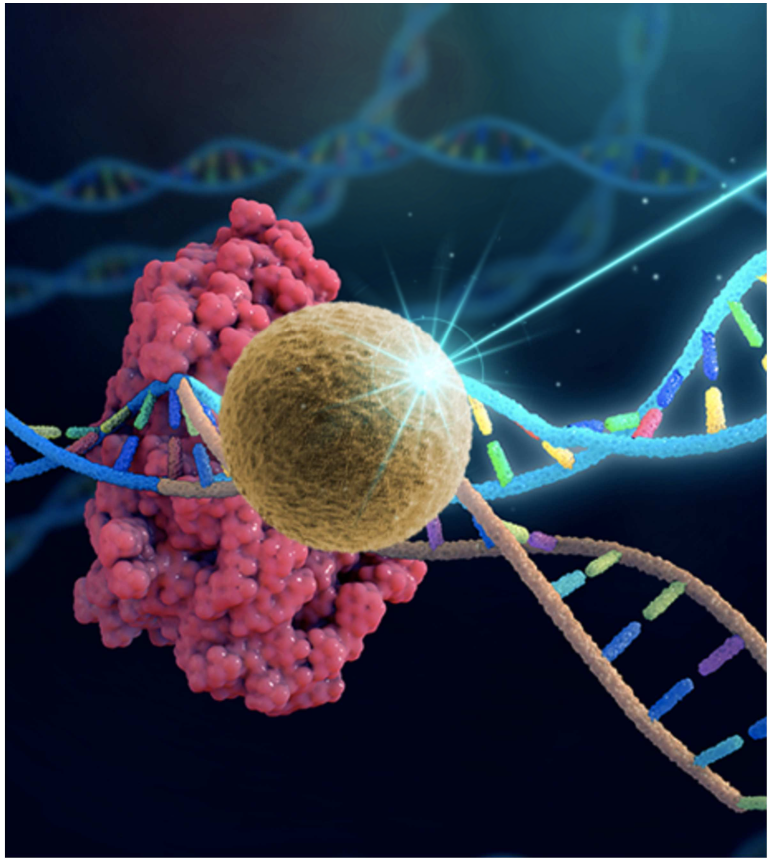
THE I-GENE PROJECT

In-vivo Gene Editing by NanotransducErs



1. A nanoparticle (NP) is incorporated in the enzyme formulation

2. Nanoparticle acts as a nanotransducer

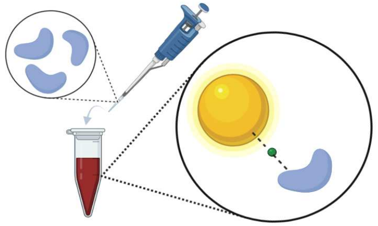
3. Upon laser absorption NP undergoes a localized temperature increase

4. Temperature increase triggers the scissor operation and DNA cleavage occurs

5. Negligible temperature increase should occur outside the enzyme-concerned volume

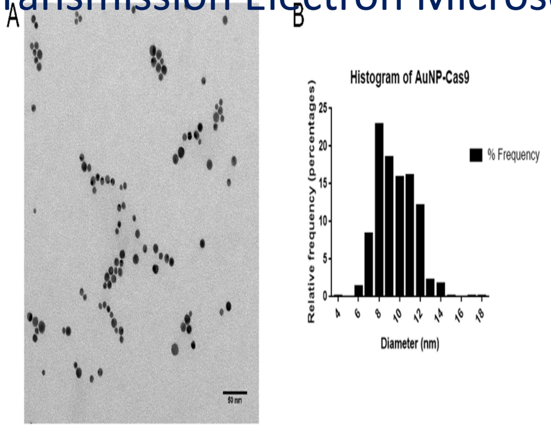
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862714.
https://i-geneproject.eu

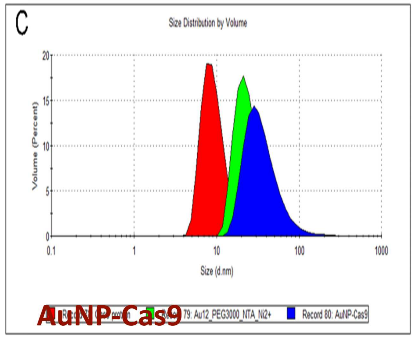
THE COMPLEX



Au spherical nanoparticles (AuNP) functionalized by affinity binding

Transmission Electron Microscopy (TEM)

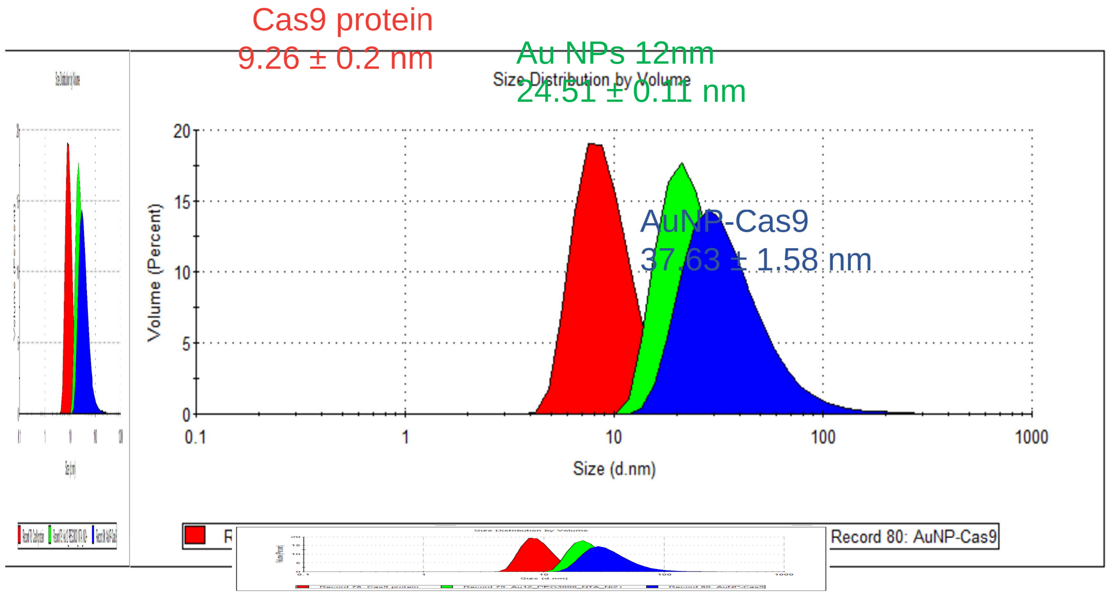




AuNP-Cas9

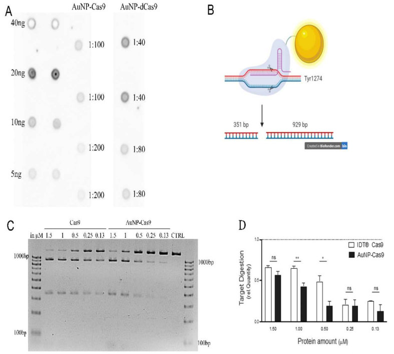
	Size (d.nm):	St Dev (d.nm):
Cas9 protein	9,264	3,036
Au12_PEG3000_NTA_Ni2+	24,37	9,057
AuNP-Cas9	37,63	23,89

Dynamic Light Scattering (DLS)

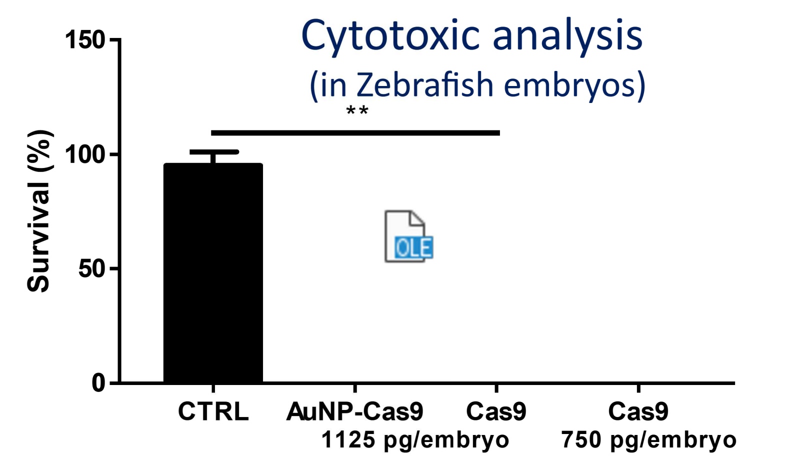


Complex effectively synthesized

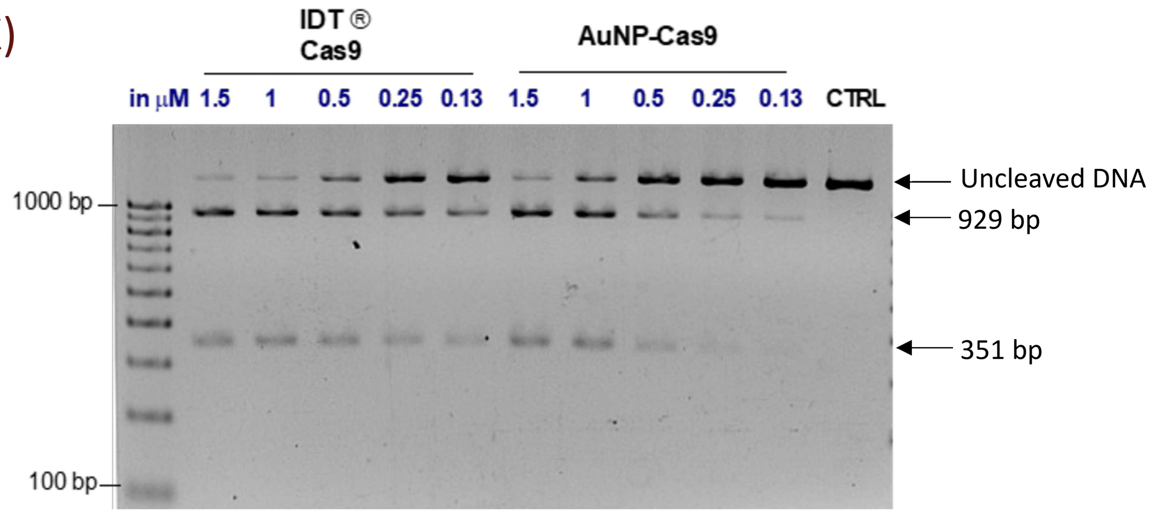
THE COMPLEX

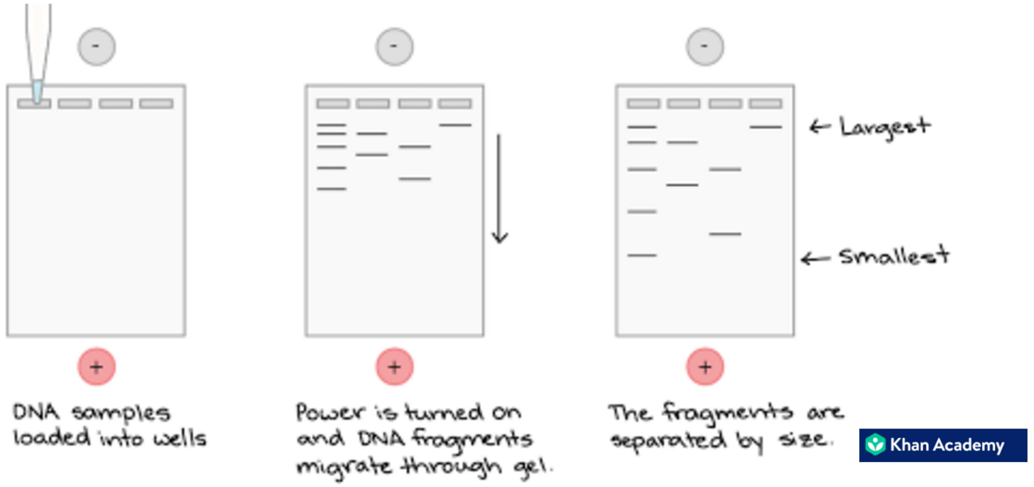


Expected enzyme activation temperature: 57 °C ($\Delta T \sim 20$ K)



Electrophoresis gel analysis





Scissors still work and the complex is viable (despite NPs)

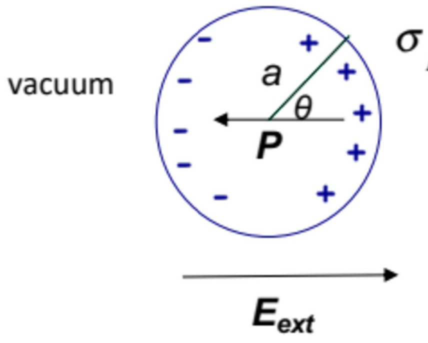
3. PLASMONICS:
HOW CAN IT HELP?

LOCALIZED PLASMON RESONANCES

STATIC POLARIZABILITY OF A DIELECTRIC SPHERE

Let's consider a dielectric, rather than metal, sphere immersed in a vacuum: we will see that the result can be exported to the metal case with no major change

The problem considered here is thus the calculation of polarizability according to Clausius-Mossotti (aka Lorentz-Lorenz) formula



$$\sigma_{pol} = -P\cos\theta$$

$$E_{loc,z} = -\frac{1}{4\pi\varepsilon_0}\int_S \frac{\sigma_{pol}\cos\theta}{a^2}\,dS$$

with $dS = 2\pi a^2 \sin\theta\, d\theta$

$$E_{loc,z} = \frac{P}{2\varepsilon_0}\int_0^\pi \cos^2\theta \sin\theta\, d\theta = \frac{P}{3\varepsilon_0} \rightarrow \vec{E}_{loc} = \frac{\vec{P}}{3\varepsilon_0}$$

$$\vec{E} = \vec{E}_{ext} + \vec{E}_{loc} = \vec{E}_{ext} + \frac{\vec{P}}{3\varepsilon_0}$$

By definition: $\vec{P} = \varepsilon_0 \chi \vec{E}_{ext} = \varepsilon_0(\varepsilon_r - 1)\vec{E}_{ext}$

By definition: $\vec{P} = \frac{N_{dip}}{Vol}\vec{p} = \frac{N_{dip}}{\frac{4}{3}\pi a^3}\vec{p}$

For an elementary dipole at the center: $\vec{p} = \alpha \vec{E}$

$$\vec{P} = \frac{N_{dip}}{\frac{4}{3}\pi a^3}\alpha\left(\vec{E}_{ext} + \frac{\vec{P}}{3\varepsilon_0}\right) = \varepsilon_0(\varepsilon_r - 1)\vec{E}_{ext}$$

$$\rightarrow \frac{N_{dip}}{\frac{4}{3}\pi a^3}\vec{E}_{ext}\alpha\left(1 + \frac{\varepsilon_0(\varepsilon_r - 1)}{3\varepsilon_0}\right) = \varepsilon_0(\varepsilon_r - 1)\vec{E}_{ext}$$

$$\Rightarrow \alpha \propto \frac{\varepsilon_r - 1}{\varepsilon_r + 2}$$

LOCALIZED PLASMON RESONANCES

Ottaviano Fabrizio Mossotti

Da Wikipedia, l'enciclopedia libera.

Ottaviano Fabrizio Mossotti (Novara, 17 aprile 1791 – Pisa, 20 marzo 1863) è stato un matematico, fisico, astronomo e accademico italiano.

ıbility according to Clausius-Mossotti

LOCALIZED PLASMON RESONANCES

DRUDE MODEL FOR THE METAL

Drude model (classical, but its main results are in general agreement with quantum models):

$$\gamma = \frac{1}{\tau_c} = \frac{ne^2}{m\sigma_c} = \frac{\varepsilon_0 \omega_p^2}{\sigma_c}$$

*Electrons in the metal undergo collisions with the lattice, giving rise to **a damping** at rate γ*

Electrons in a metal driven by an oscillating electric field are well described by a damped/driven motion:

$$m\frac{d^2\vec{r}}{dt^2} + \gamma\frac{d\vec{r}}{dt} = -e\vec{E}$$

$$\vec{E} = \vec{E}(t) = \vec{E}_0 \exp(-i\omega t) \rightarrow \vec{r} = \vec{r}(t) = \vec{r}_0 \exp(-i\omega t)$$

$$\rightarrow -\omega^2 m\vec{r}_0 - i\omega\gamma m\vec{r}_0 = -e\vec{E}_0$$

$$\rightarrow \vec{r}_0 = \vec{E}_0 \frac{e}{m\omega^2 + i\omega\gamma m}$$

The polarization field can be written as:

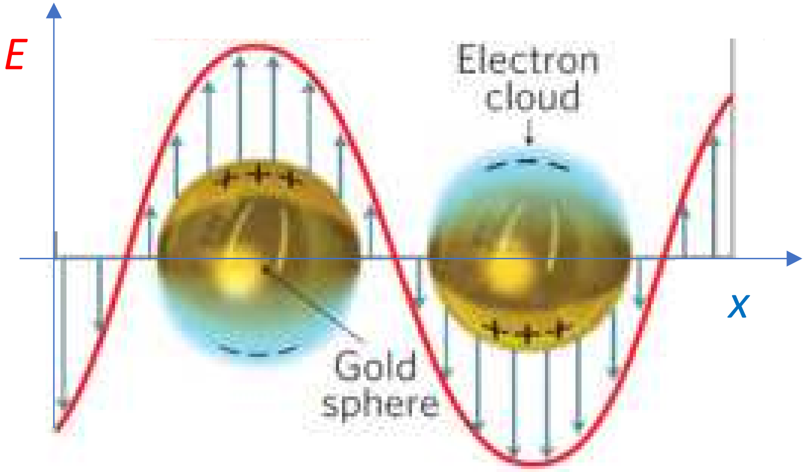
$$\vec{P} = -ne\vec{r} = -\frac{ne^2}{m\omega^2 + i\omega\gamma m}\vec{E} = -\varepsilon_0\frac{\omega_p^2}{\omega^2 + i\gamma\omega}\vec{E} = \chi\vec{E}$$

Drude model leads to a frequency dependent, complex dielectric constant describing the metal

$$\chi = -\frac{\omega_p^2}{\omega^2 + i\gamma\omega} \rightarrow \varepsilon_r = 1 + \chi = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma\omega}$$

LOCALIZED PLASMON RESONANCES

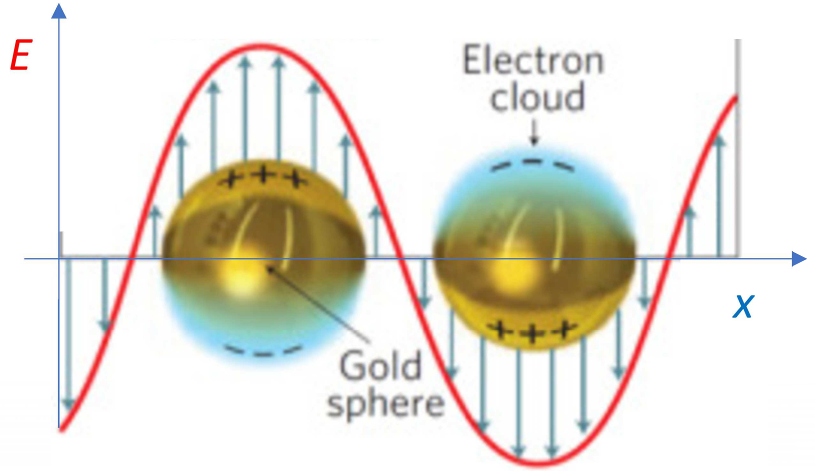
$$\alpha = 4\pi\varepsilon_0 a^3 \frac{\varepsilon_{metal} - \varepsilon_{dielectric}}{\varepsilon_{metal} + 2\varepsilon_{dielectric}}$$



Lycurgus cup (IV century AD) @ British Museum

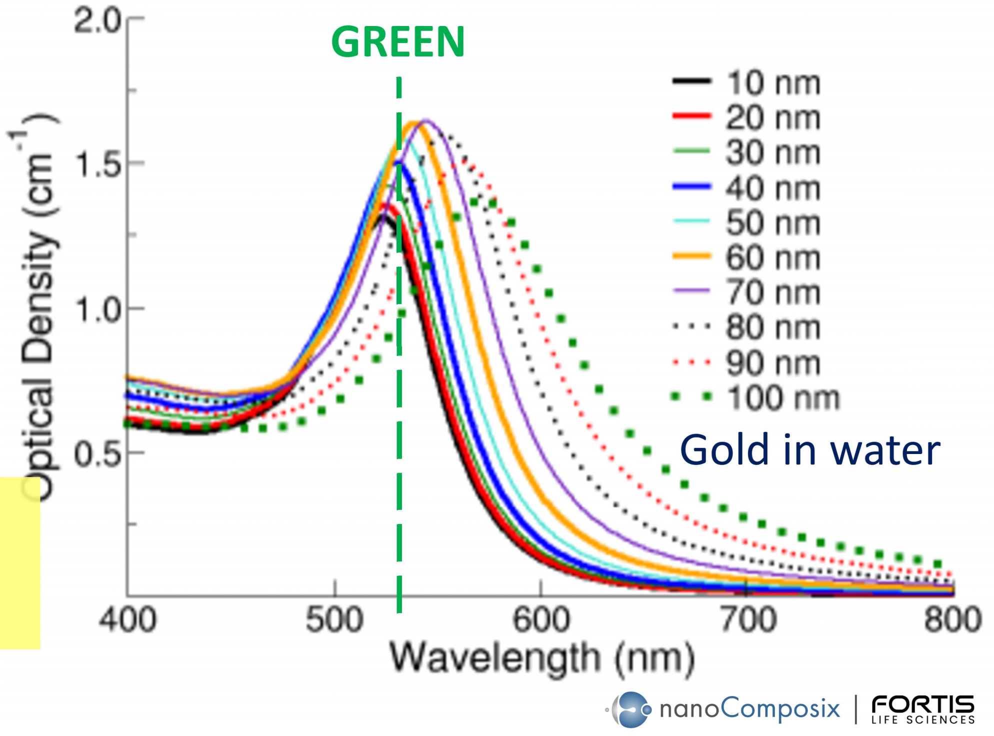
LOCALIZED PLASMON RESONANCES

$$\alpha = 4\pi\varepsilon_0 a^3 \frac{\varepsilon_{metal} - \varepsilon_{dielectric}}{\varepsilon_{metal} + 2\varepsilon_{dielectric}}$$



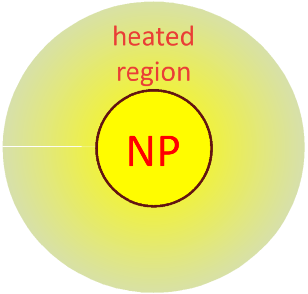
Lycurgus cup (IV century AD) @ British Museum

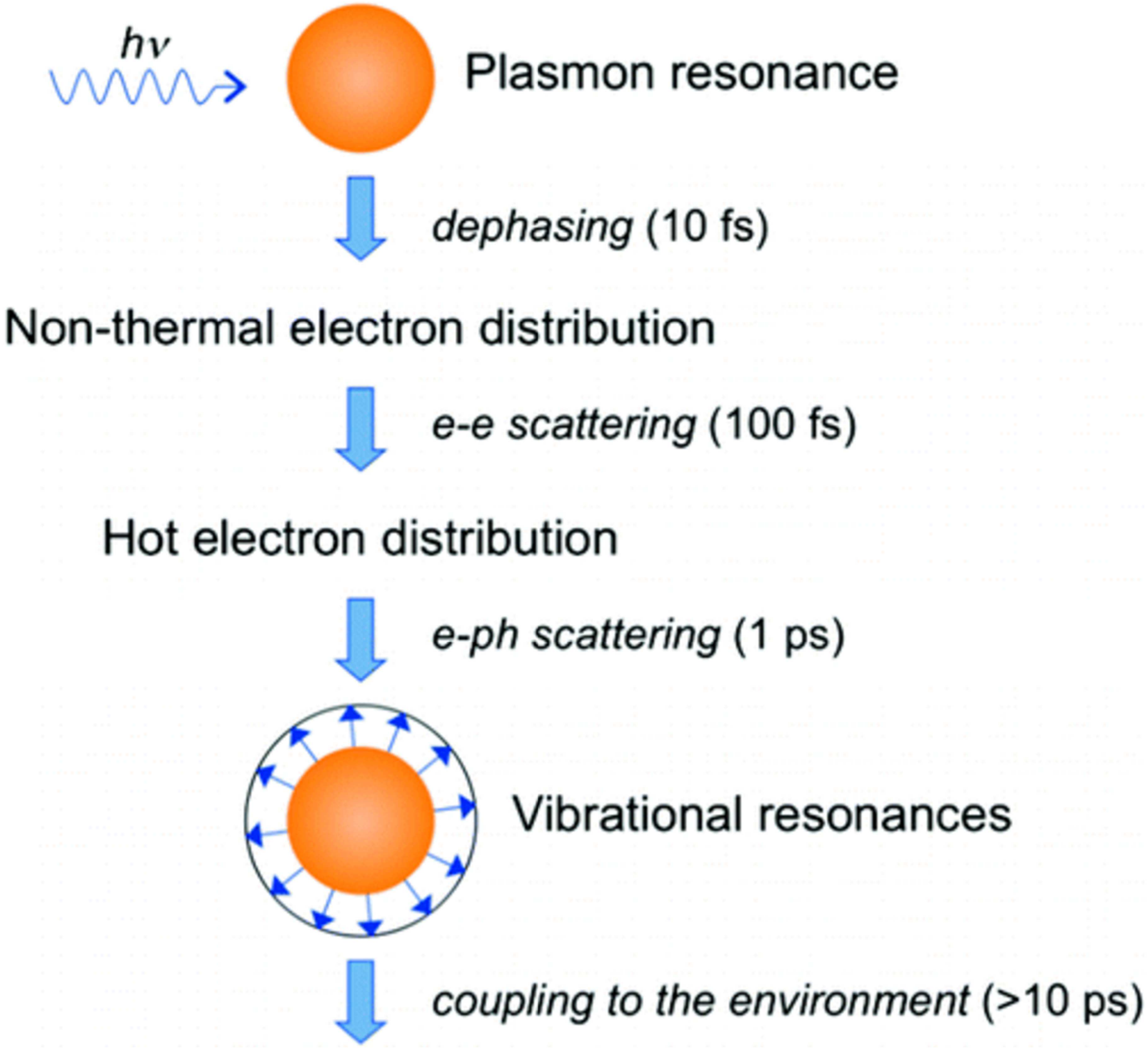
Resonance leads to enhanced absorption of light



SIMULATED HEATING

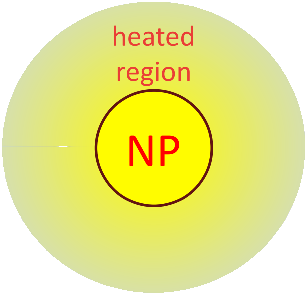
Multiphysics simulation including light absorption, temperature increase, heat propagation



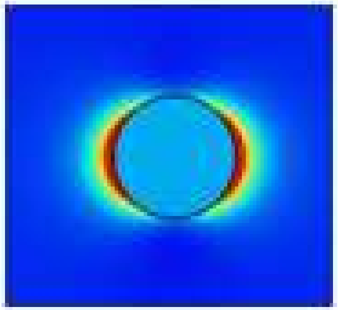


SIMULATED HEATING

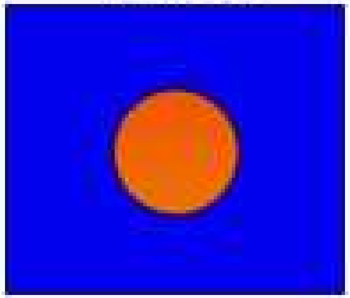
Multiphysics simulation including light absorption, temperature increase, heat propagation



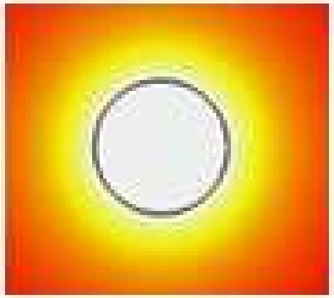
Electric field amplitude

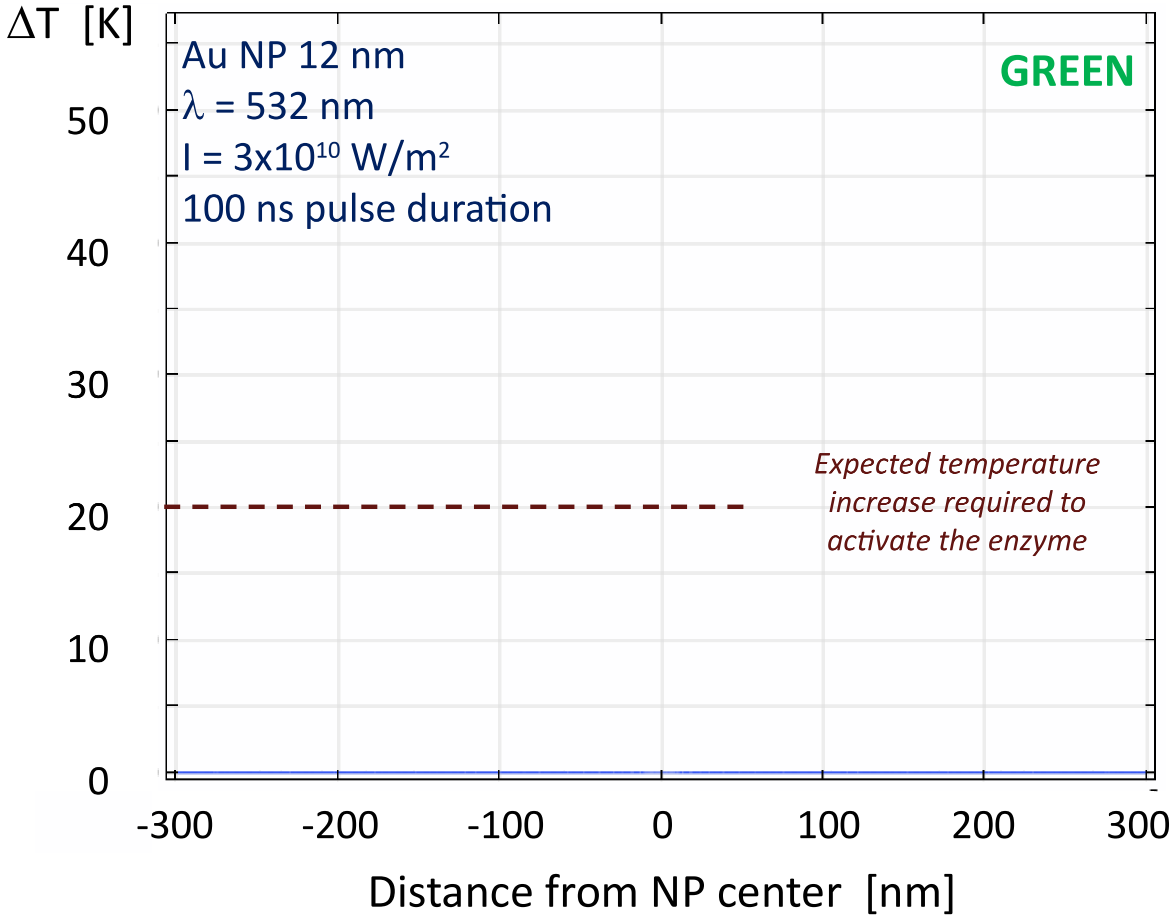


Power absorption



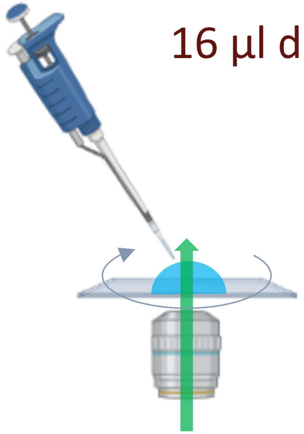
Temperature increase





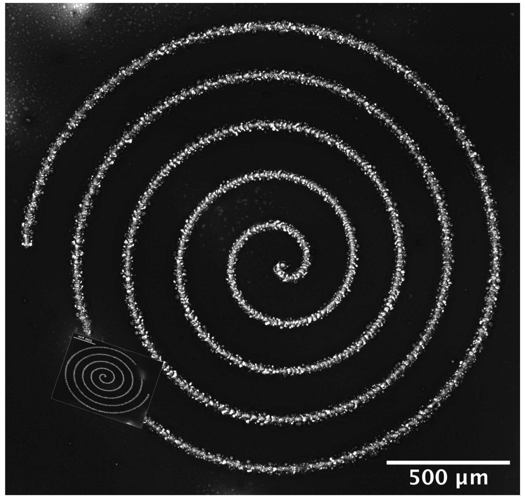
Experimentally, we should be in the proper range

EXPERIMENT

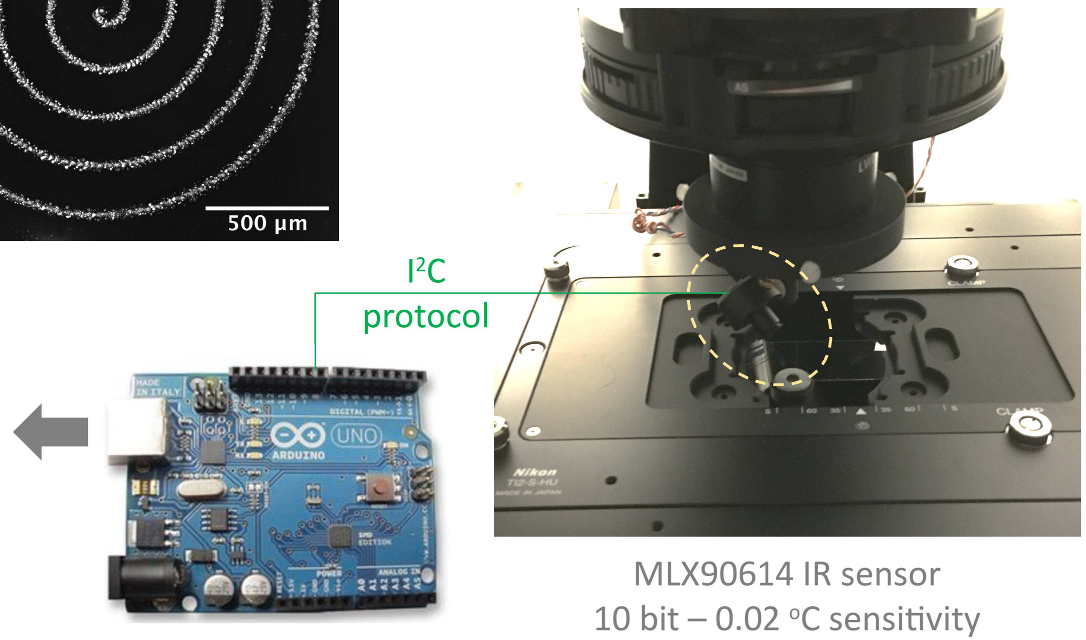


16 µl drop (4.5 mm diameter) containing DNA sample in solution

Spiral-like trajectory to increase irradiated area (60 s irradiation)

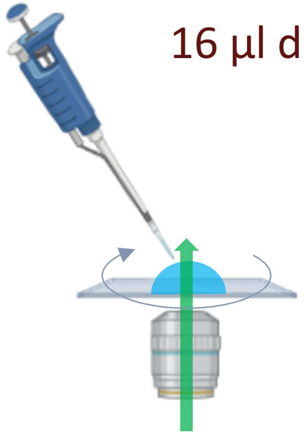


Real-time monitor of the bulk temperature



MLX90614 IR sensor
10 bit – 0.02 °C sensitivity

EXPERIMENT



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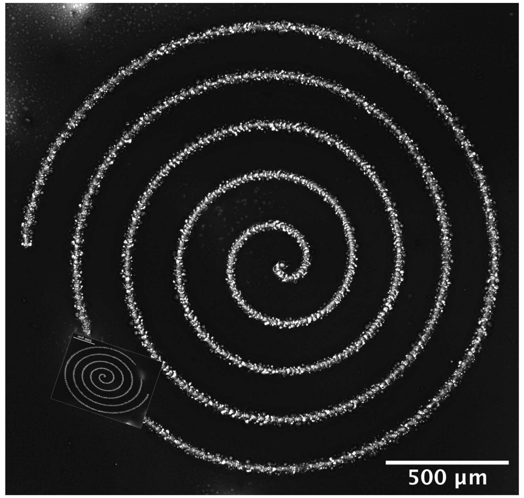
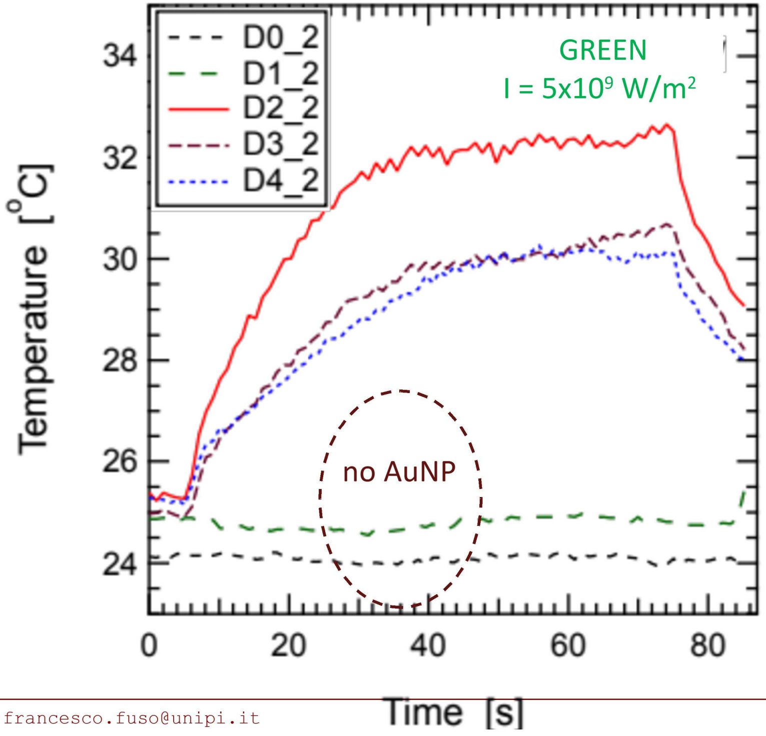
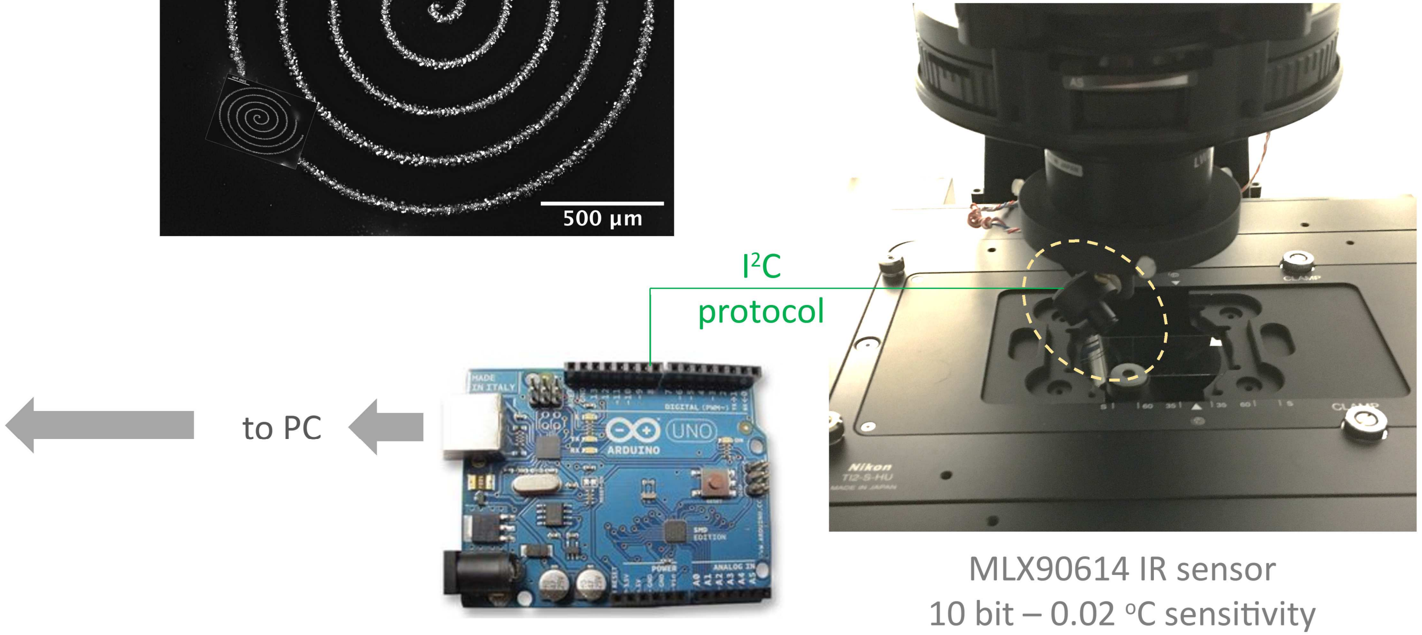


Real-time monitor of the bulk temperature



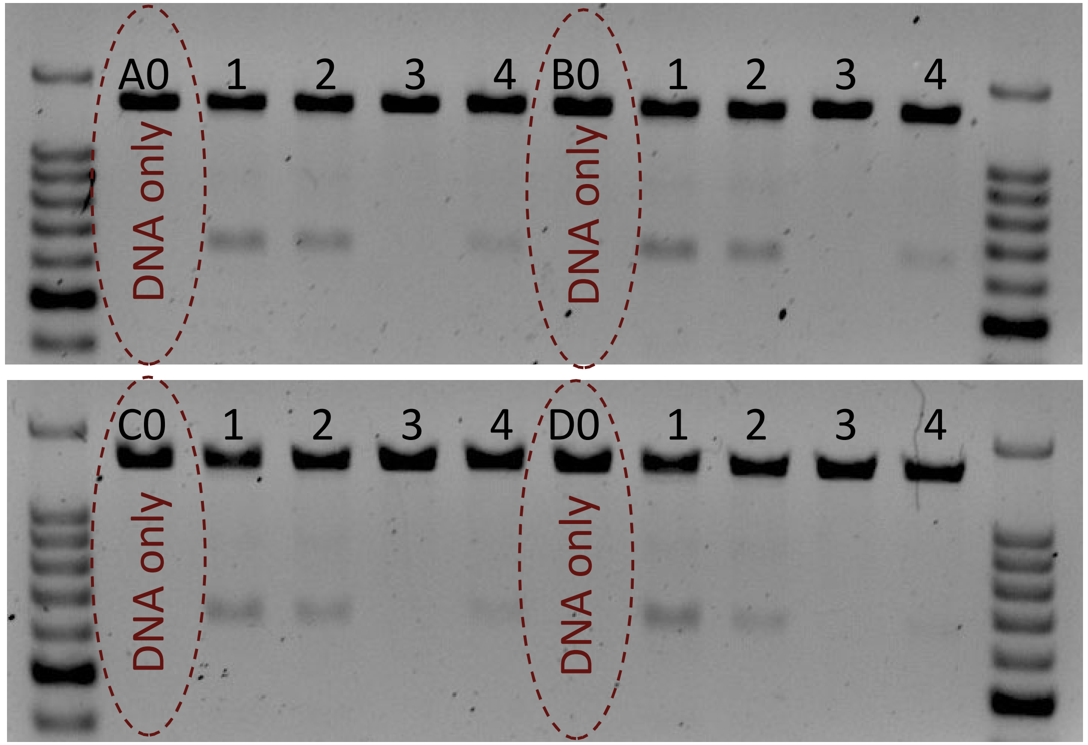


MLX90614 IR sensor

10 bit – 0.02 °C sensitivity

PRELIMINARY

Electrophoresis gel analysis



Laser intensity

A : 5×10^9 W/m^2
B : 2.5×10^9 W/m^2
C : 1×10^9 W/m^2
D : 0.6×10^9 W/m^2

Sample material legend

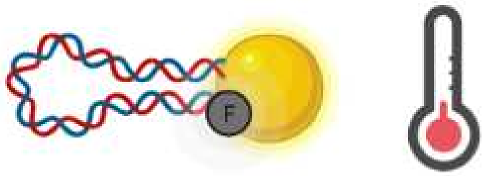
0 : DNA only
1 : DNA + RE
2 : DNA + AuNP + RE + dCas9
3 : DNA + AuNP + dCas9 (functionalized)
4 : DNA + AuNP + RE + dCas9 (functionalized)

Not properly working, yet!

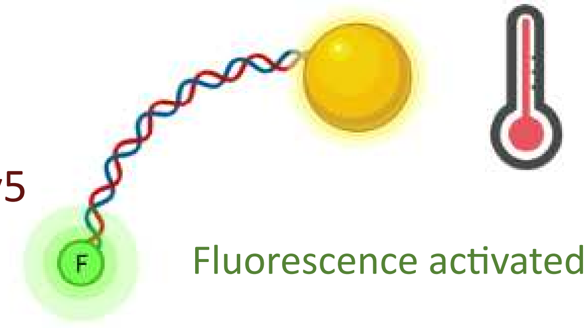
LOCAL TEMPERATURE INCREASE

DNA hairpin functionalized
with a fluorophore

A nanosized thermometer



Fluorescence quenched

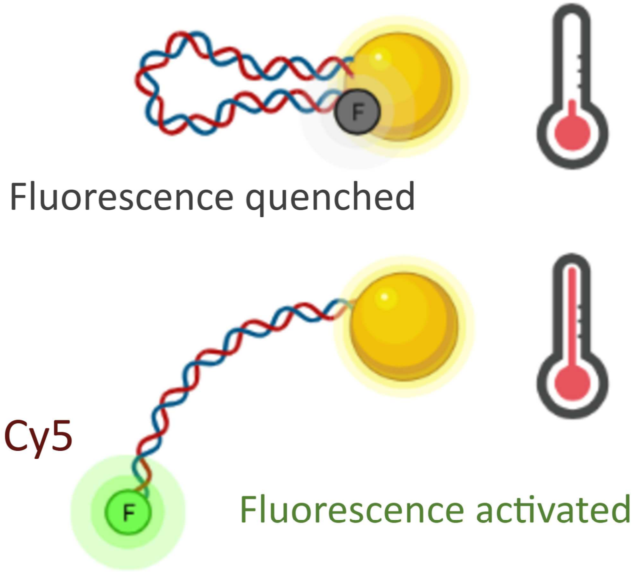


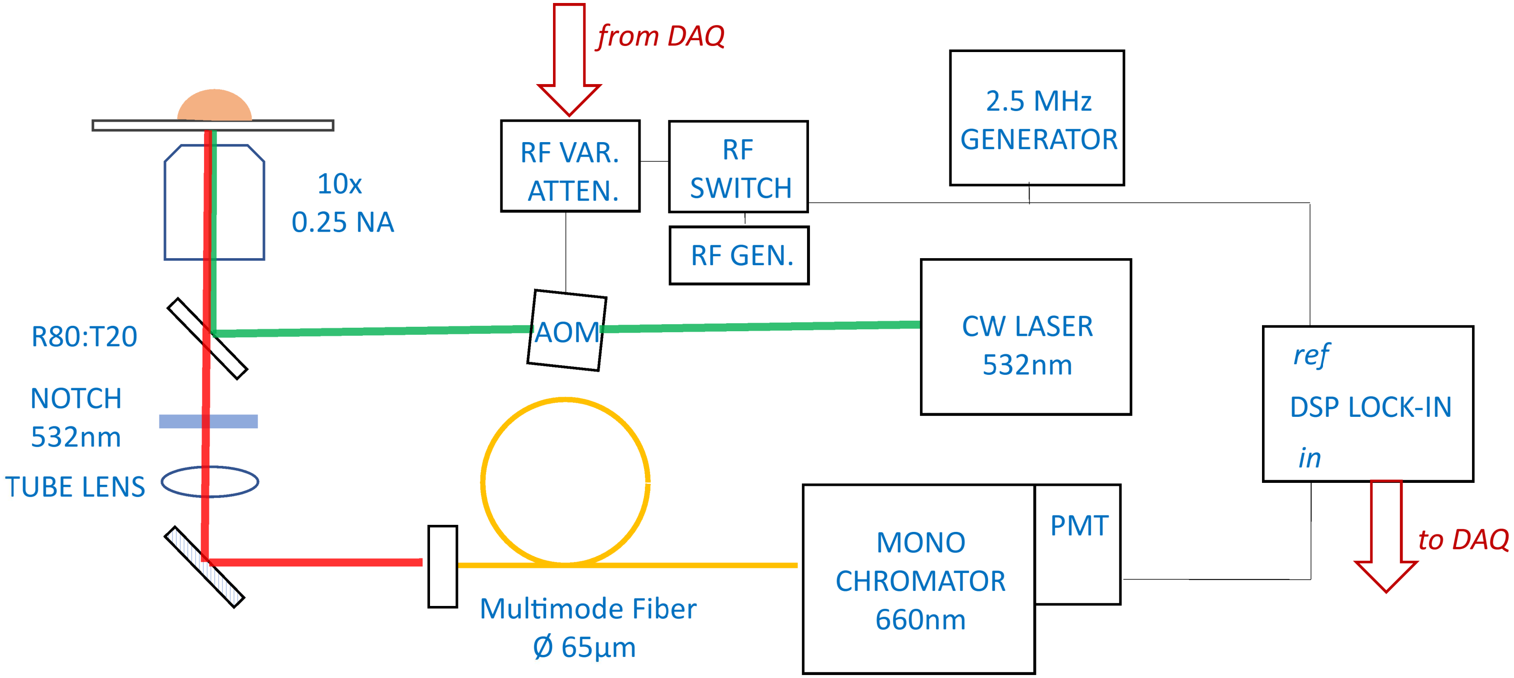
Cy5

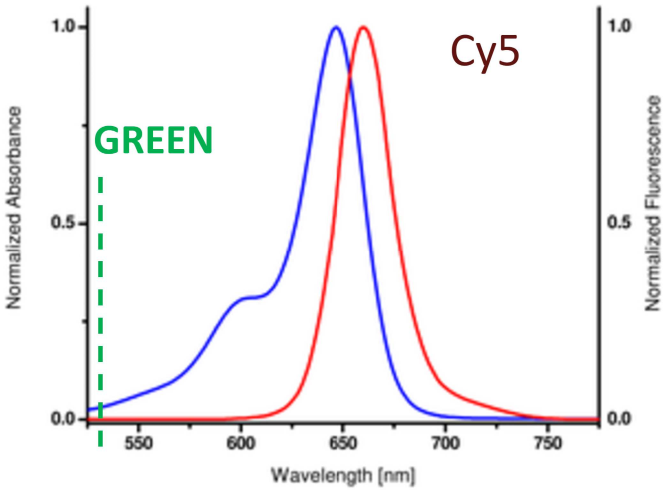
Fluorescence activated

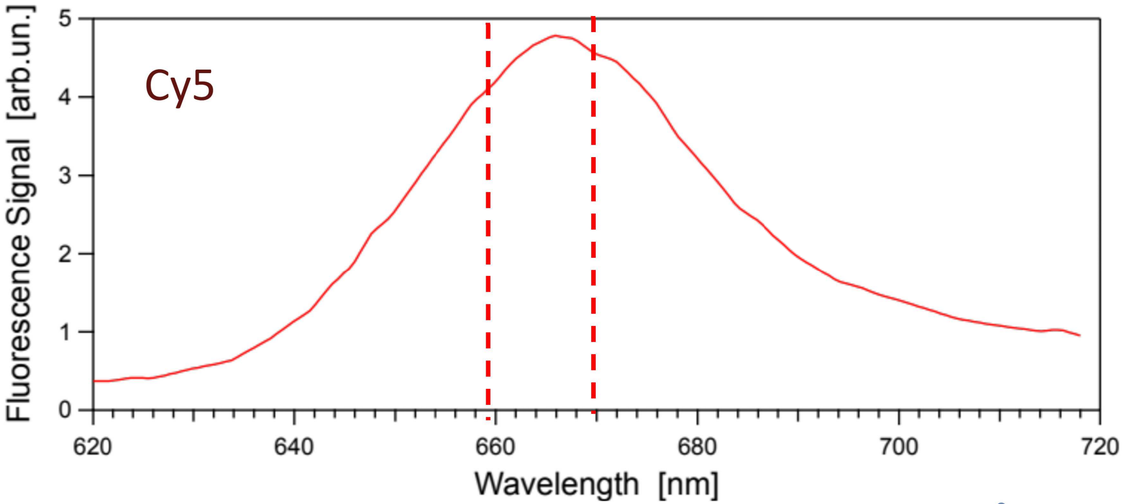
LOCAL TEMPERATURE INCREASE

DNA hairpin functionalized with a fluorophore

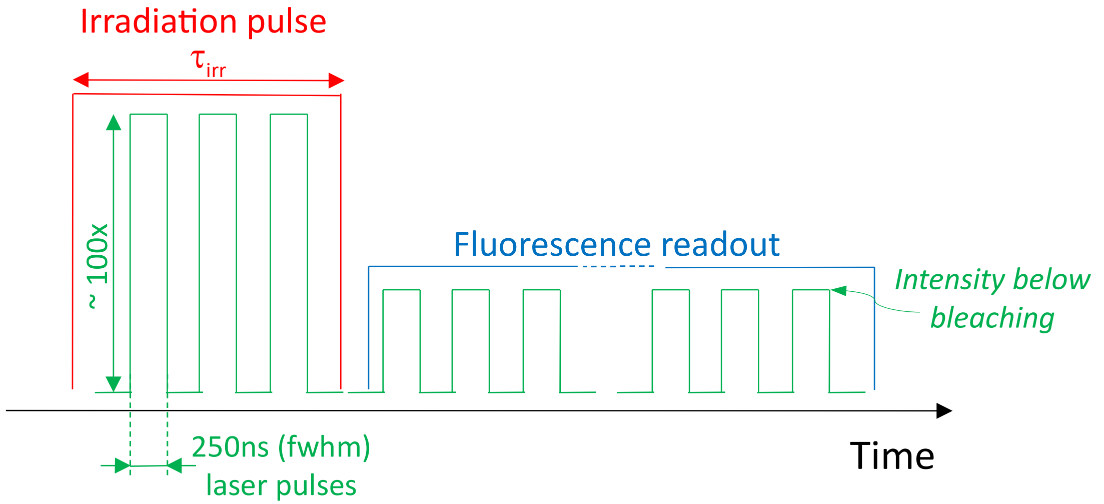


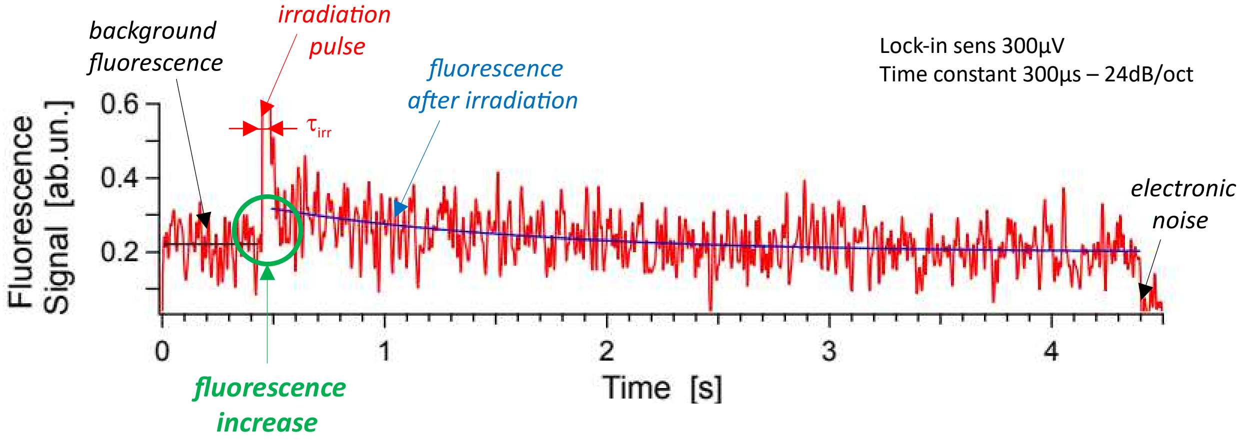






LOCAL TEMPERATURE INCREASE





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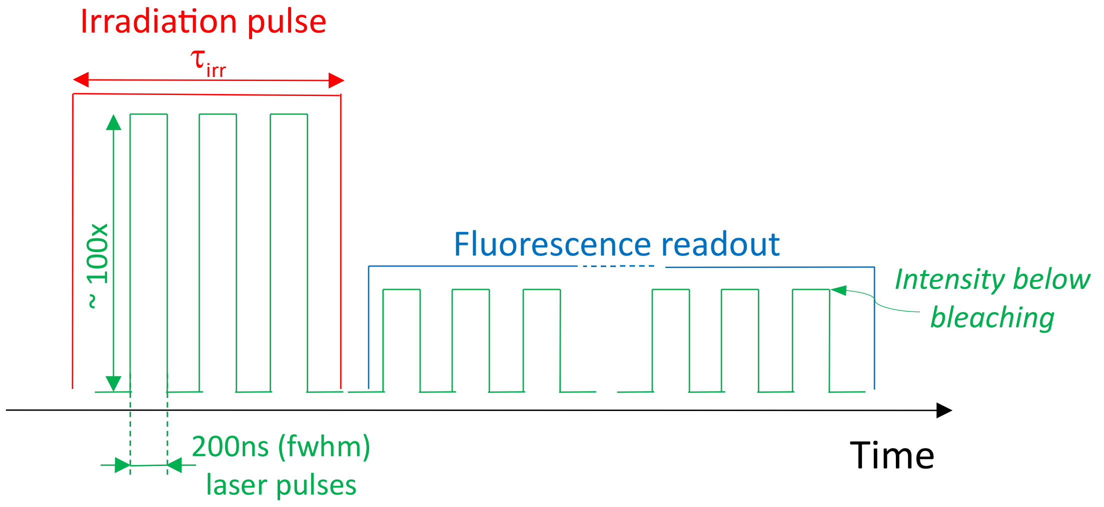
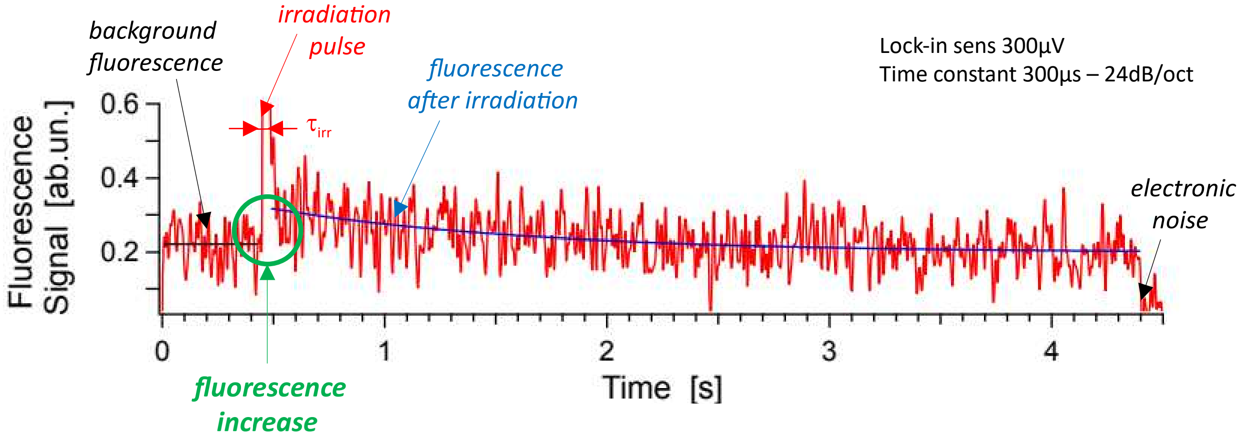
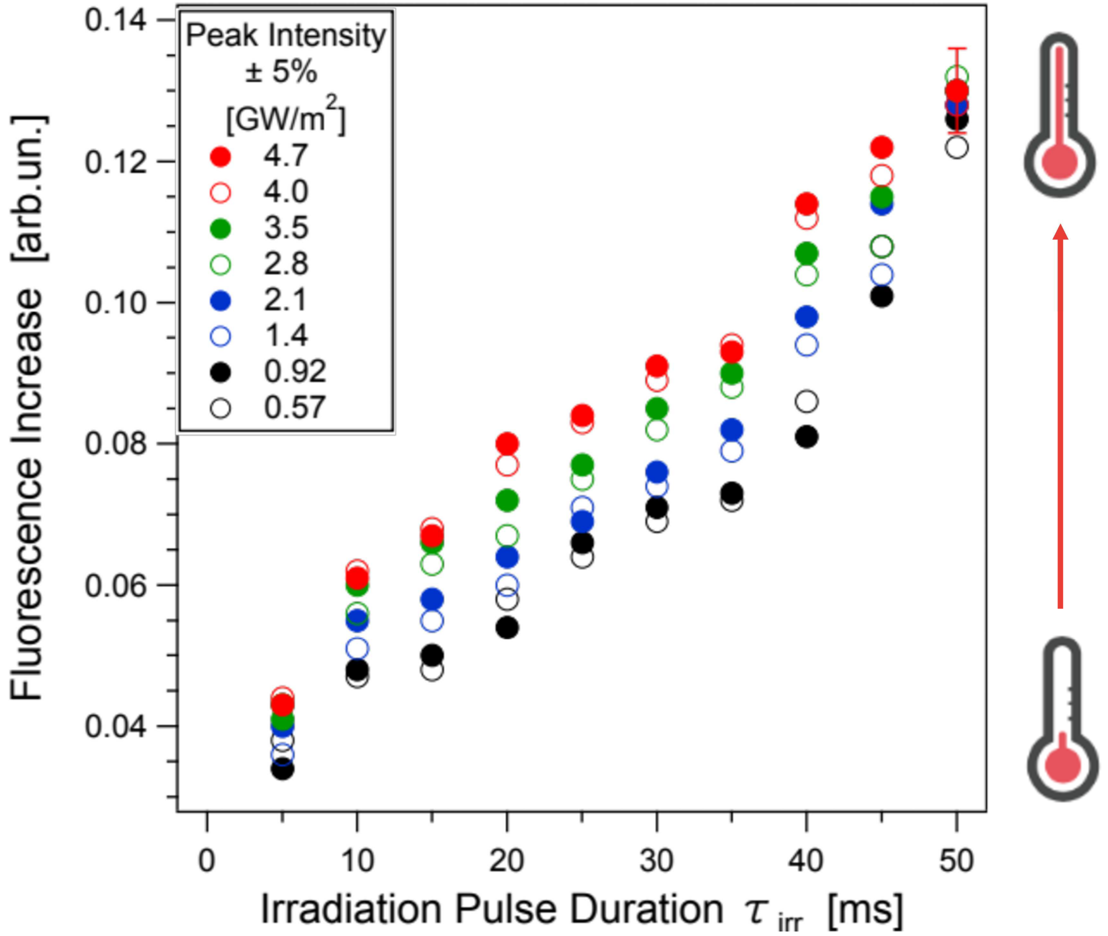
LOCAL TEMPERATURE INCREASE





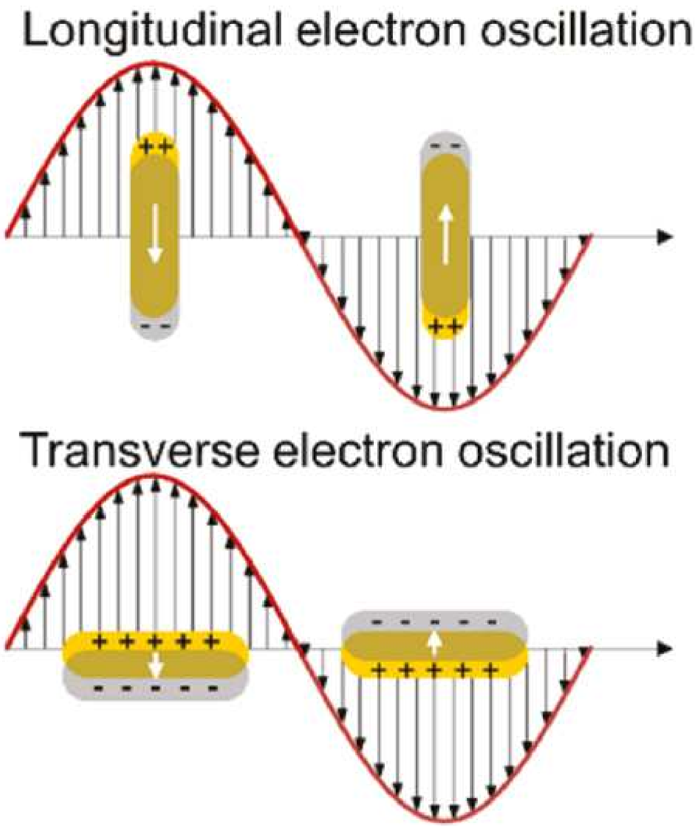


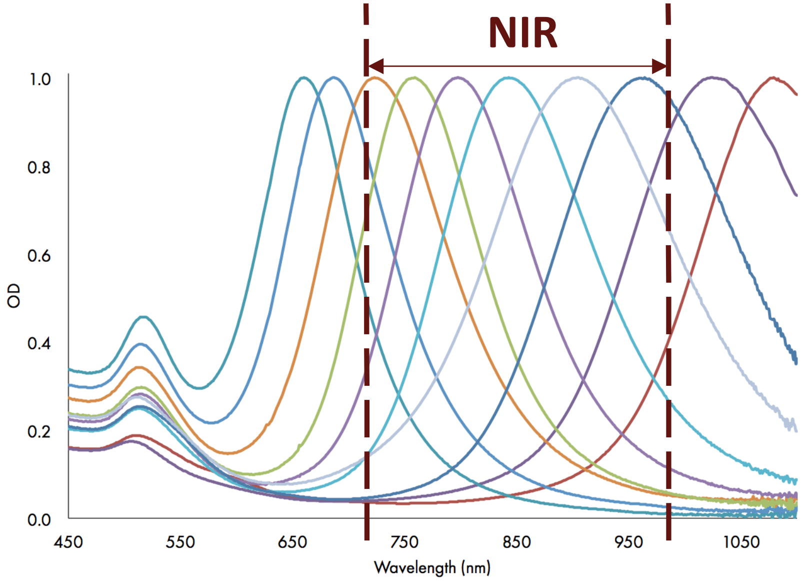
Temperature increase confirmed

3bis. PLASMONICS: ANOTHER STRATEGY

Au NANORODS

Using nanorods rather than nanospheres leads to another, red-shifted, plasmon resonance band





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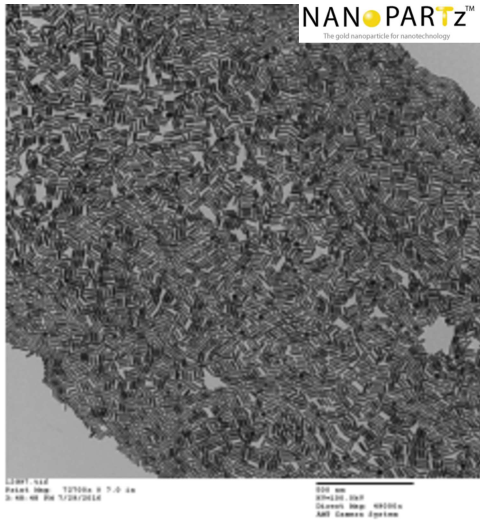
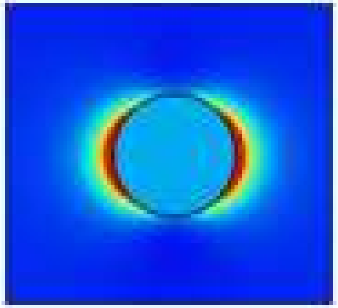


https://doi.org/10.1515/nanoph-2017-0064

DIMERS

Electric field amplitude

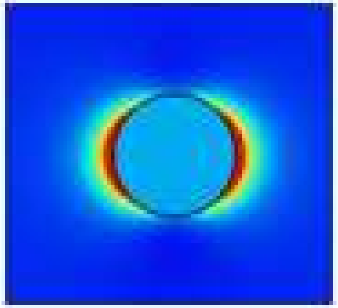


Field amplitude strongly enhanced close to the NP surface

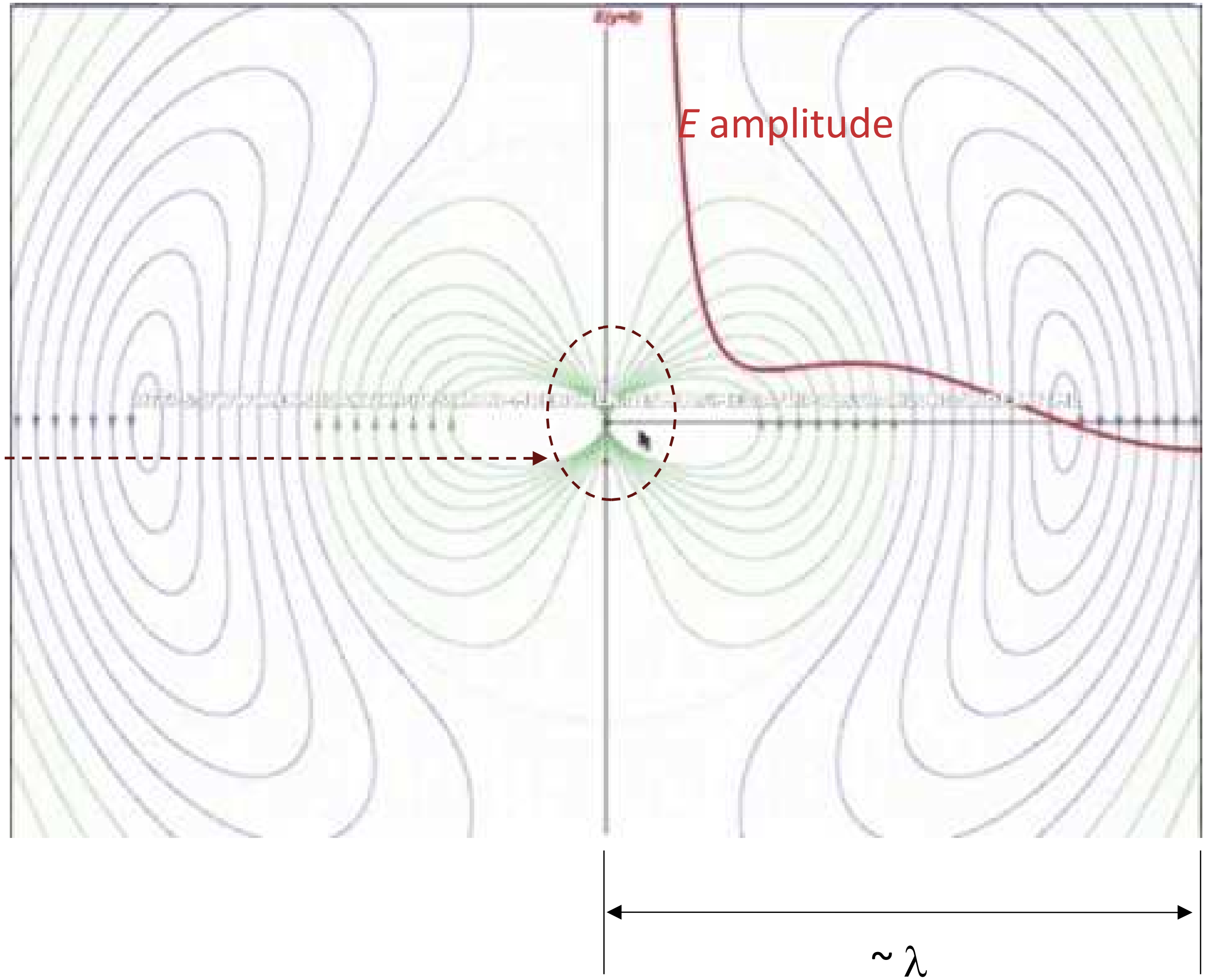
Near-field effect

DIMERS

Electric field amplitude

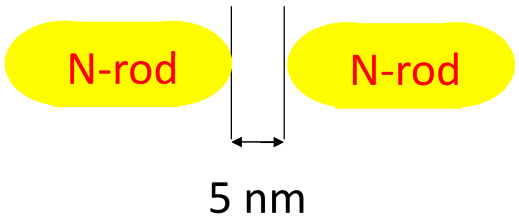


Oscillating dipole

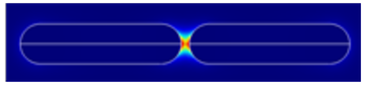


http://iopscience.iop.org/article/10.1088/0143-0807/37/6/065206/meta

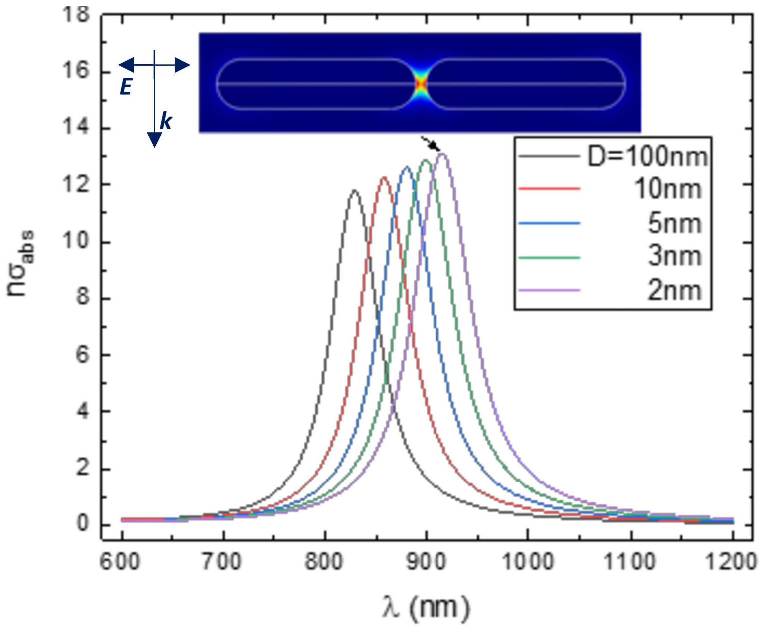
DIMERS

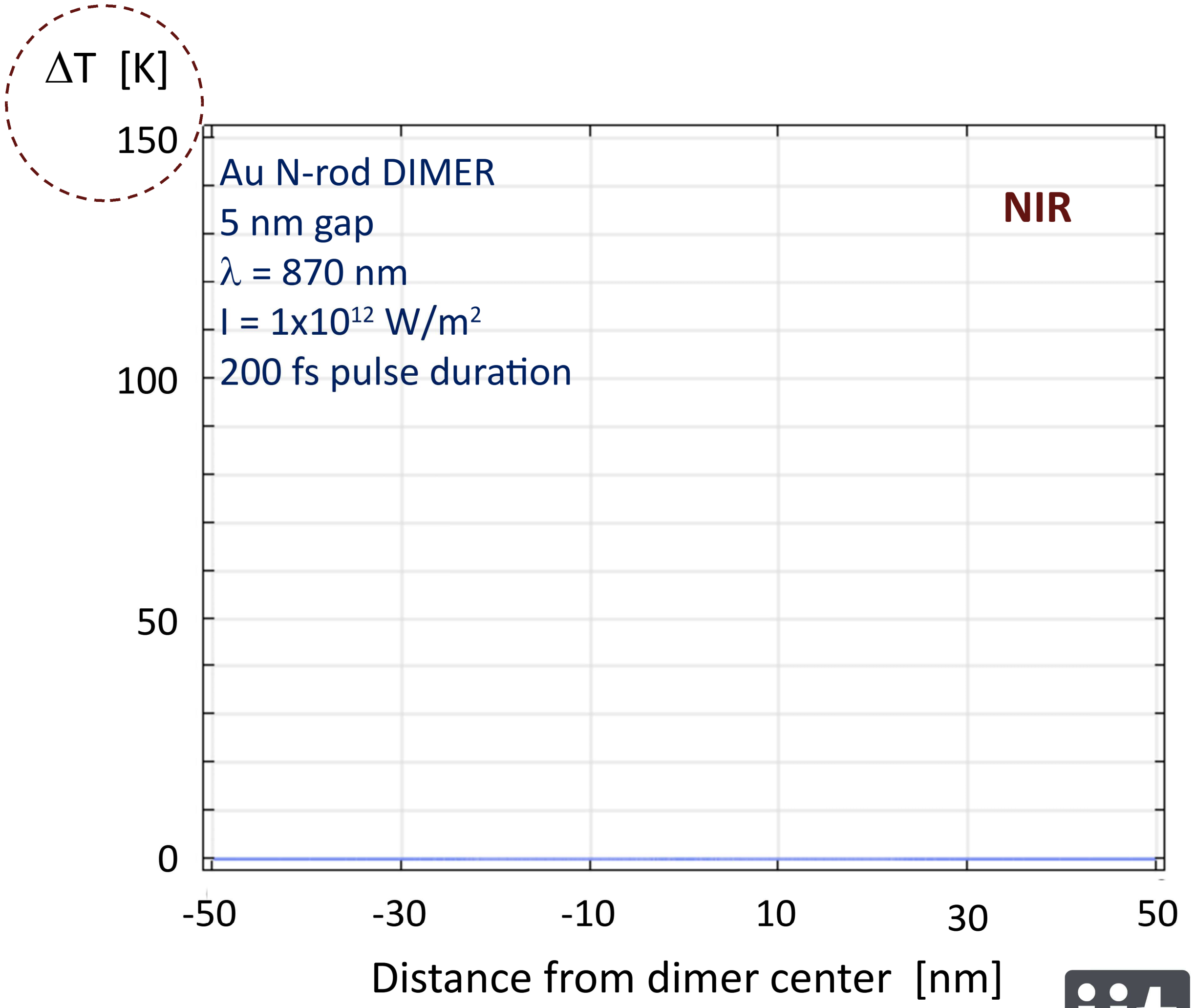


Electric field amplitude

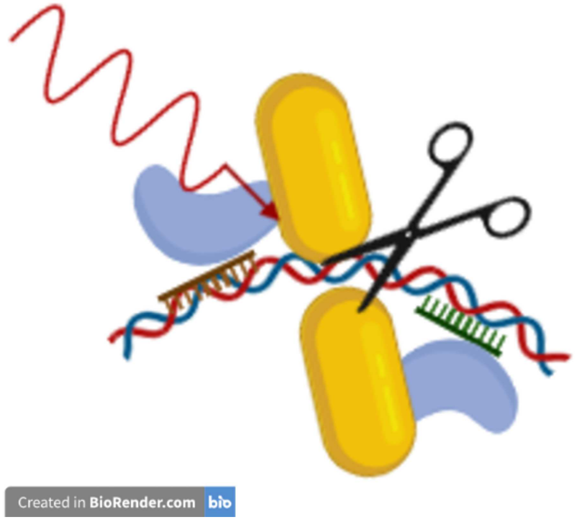


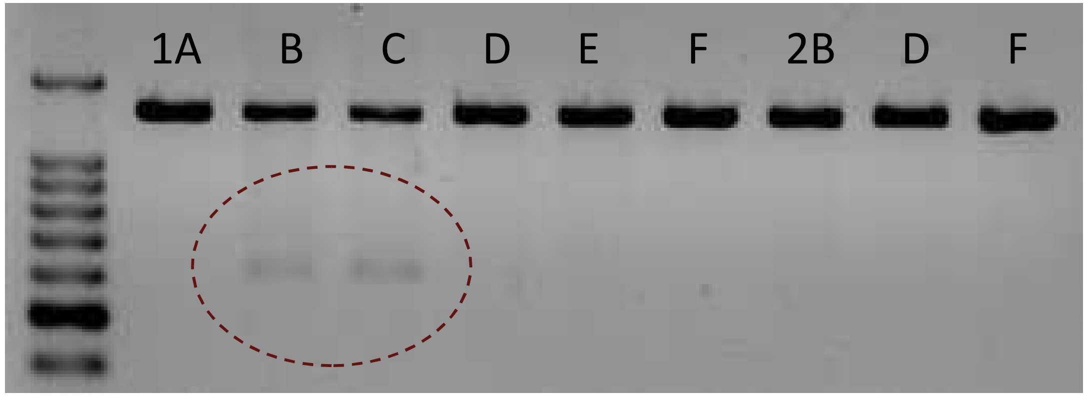
Temperature increase at two subsequent times ($t_2 = t_1$+100 fs)





PRELIMINARY



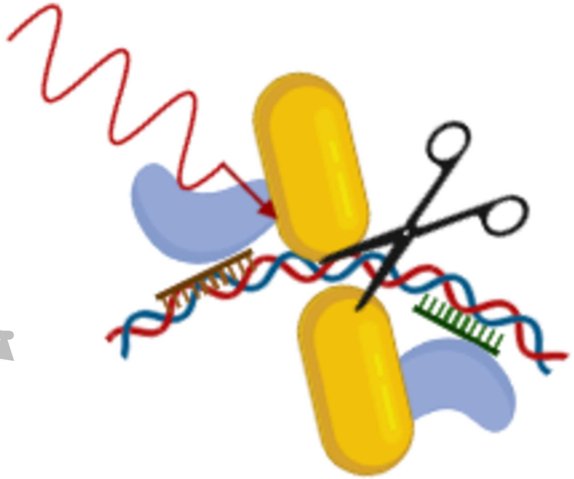


Laser intensity
1 : $8x10^{14}$ W/m^2
2 : $4x10^{14}$ W/m^2

Sample material legend
A : only DNA
B : DNA + dimer @$15x10^{10}$
C : DNA + dimer @$30x10^{10}$
D : DNA + dimer @$7.5x10^{10}$
E : DNA + dimer @$5x10^{10}$
F : DNA + dimer @$2.5x10^{10}$

Maybe!

CONCLUSIONS



- An experimental setup has been developed
- Simulations suggest feasibility
- Preliminary in-vitro tests in progress
- Still lot of work to do including other directions

I·Gene

The presented project is part of the I-GENE project, which is funded by Horizon 2020, Call identifier: H2020-FETOPEN-2018-2020

I-

Partners

I-GENE Consortium is composed of 5 partners (2 from Accademia and 3 from Industry), recognized for excellence in the field of gene therapy, nanomedicine, photonics and material science.

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